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(21) International Application Number: PCT/US97/14347 (22) International Filing Date: 15 August 1997 (15.08.97) (30) Priority Data: <table border="0"><tr><td>08/858,985</td><td>27 May 1997 (27.05.97)</td><td>US</td></tr><tr><td>08/863,624</td><td>27 May 1997 (27.05.97)</td><td>US</td></tr><tr><td>08/864,545</td><td>28 May 1997 (28.05.97)</td><td>US</td></tr><tr><td>08/900,194</td><td>25 July 1997 (25.07.97)</td><td>US</td></tr></table> (71) Applicant: GUILFORD PHARMACEUTICALS INC. [US/US]; 6611 Tributary Street, Baltimore, MD 21224 (US). (72) Inventors: JACKSON, Paul, F.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). SLUSHER, Barbara, S.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). TAYS, Kevin, L.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). MACLIN, Keith, M.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). (74) Agent: NATH, Gary, M.; Nath & Associates, 1030 15th Street, N.W., 6th Floor, Washington, DC 20005 (US).	08/858,985	27 May 1997 (27.05.97)	US	08/863,624	27 May 1997 (27.05.97)	US	08/864,545	28 May 1997 (28.05.97)	US	08/900,194	25 July 1997 (25.07.97)	US	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
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(54) Title: INHIBITORS OF NAALADASE ENZYME ACTIVITY (57) Abstract <p>The present disclosure relates to dipeptidase inhibitors, and more particularly, to novel methods of using phosphonate derivatives, hydroxyphosphinyl derivatives, and phosphoramidate derivatives to inhibit N-Acetylated α-Linked Acidic Dipeptidase (NAALADase) enzyme activity, and to treat prostate diseases, especially using the compounds of the present invention for the inhibition of the growth of prostate cancer cells.</p>													

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INHIBITORS OF NAALADASE ENZYME ACTIVITY

RELATED APPLICATIONS

5 This application is a continuation-in-part (CIP) of U.S. patent application filed May 28, 1997, entitled "Methods of Cancer Treatment Using NAALADase Inhibitors", which is a CIP of U.S. patent application serial number 08/665,775, filed June 17, 1996, entitled "Methods of Cancer Treatment Using NAALADase Inhibitors"; U.S. patent application filed May 27, 10 1997, entitled "NAALADase Inhibitors", which is a CIP of U.S. patent application serial number 08/665,776, filed June 17, 1996, entitled "NAALADase Inhibitors", and U.S. patent application filed May 27, 1997, entitled "NAALADase Inhibitors", which is also a CIP of U.S. patent application 15 serial number 08/665,776.

BACKGROUND OF THE INVENTION1. Field of the Invention

20 The present invention relates to novel NAALADase inhibitors and methods of using the same for treating cancer, preventing tumor cell growth, inhibiting prostate tumor cell growth, and inhibiting NAALADase enzyme activity, by administering an effective amount of the NAALADase inhibitor.

2. Description of the Prior Art25 Prostate Cancer

Prostate cancer is the leading form of cancer and the second leading cause of death from cancer for men in the United States. The American Cancer Society has estimated that in 1996 alone, 317,100 new cases of prostate cancer were

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diagnosed and 41,400 deaths were caused by prostate cancer. The incidence rate of prostate cancer increased 65% between 1980 and 1990, and will continue to rise with improved screening tests and longer life expectancies. While most men
5 used to die of other illnesses before prostate cancer had a chance to develop, higher prostate cancer mortality rates are expected as men live longer and the disease has more time to progress.

In 1993, the molecular cloning of Prostate Specific Membrane Antigen (PSMA) was reported as a potential prostate carcinoma marker and hypothesized to serve as a target for
10 imaging and cytotoxic treatment modalities for prostate cancer. PSMA antibodies, particularly indium-111 labelled and tritium labelled PSMA antibodies, have been described and examined clinically for the diagnosis and treatment of
15 prostate cancer. PSMA is expressed in prostatic ductal epithelium and is present in seminal plasma, prostatic fluid and urine. In 1996, it was found that the expression of PSMA cDNA confers the activity of NAALADase.

NAALADase Inhibitors

20 NAAG and NAALADase have been implicated in several human and animal pathological conditions relating to glutamate abnormalities and neurotoxicity. For example, it has been demonstrated that intra-hippocampal injections of NAAG elicit
25 prolonged seizure activity. More recently, it was reported that rats genetically prone to epileptic seizures have a persistent increase in their basal level of NAALADase activity. These observations lend support the hypothesis that

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increased availability of synaptic glutamate elevates seizure susceptibility, and suggest that NAALADase inhibitors may provide anti-epileptic activity.

NAAG and NAALADase have also been implicated in the pathogenesis of ALS and in the pathologically similar animal disease called Hereditary Canine Spinal Muscular Atrophy (HCSMA). It has been shown that concentrations of NAAG and its metabolites -- NAA, glutamate and aspartate -- are elevated two- to three-fold in the cerebrospinal fluid of ALS patients and HCSMA dogs. Additionally, NAALADase activity is significantly increased (two- to three-fold) in post-mortem spinal cord tissue from ALS patients and HCSMA dogs. As such, NAALADase inhibitors might be clinically useful in curbing the progression of ALS if an increased metabolism of NAAG is responsible for the alterations of CSF levels of these acidic amino acids and peptides.

Abnormalities in NAAG levels and NAALADase activity have also been documented in post-mortem schizophrenic brain, specifically in the prefrontal and limbic brain regions.

The findings described above suggest that NAALADase inhibitors could be useful in treating glutamate abnormalities. However, the present invention is directed to the surprising and unexpected discovery that the novel compounds of the present invention are not only effective NAALADase inhibitors but are effective in treating prostate diseases, particularly prostate cancer. Although the cancer data relate to prostate cancer cells, NAALADase inhibitors are expected to be equally effective in treating cancer of other

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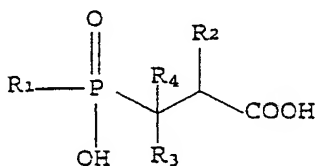
tissues where NAALADase enzyme reside, such as the brain, kidney and testis.

While a few NAALADase inhibitors have been identified, they have only been used in non-clinical research. Examples
5 of such inhibitors include general metallopeptidase inhibitors such as o-phenanthroline, metal chelators such as EGTA and EDTA, and peptide analogs such as quisqualic acid and β -NAAG. Accordingly, a need exists for more NAALADase inhibitors to be identified and, particularly, for the treatment of prostate
10 diseases such as prostate cancer.

SUMMARY OF THE INVENTION

The present invention is directed to novel NAALADase inhibitors and methods of using the same for treating cancer,
15 preventing tumor cell growth, inhibiting prostate tumor cell growth, and inhibiting NAALADase enzyme activity, in an animal, comprising administering an effective amount of the NAALADase inhibitor.

Preferred NAALADase inhibitors include compounds of
20 formula I:



I

wherein

R₁ is hydrogen, C₁-C₈ straight or branched chain alkyl,
25 C₂-C₈ straight or branched chain alkenyl group, C₃-C₈

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cycloalkyl, C₅-C₈, cycloalkenyl, or Ar₁;

R₂ is C₁-C₈, straight or branched chain alkyl, C₂-C₈, straight or branched chain alkenyl group, C₃-C₈, cycloalkyl, C₅-C₈, cycloalkenyl, or Ar₁, wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl or aryl groups may be optionally substituted with carboxylic acid;

R₃ and R₄ are independently hydrogen, C₁-C₈, straight or branched chain alkyl, C₂-C₈, straight or branched chain alkenyl, dialkyl, halogen, or Ar₁ provided that both R₃ and R₄ are not hydrogen.

Preferably, the compound of formula I is present in an amount that is effective for inhibiting NAALADase enzyme activity, or treating a prostate disease in an animal.

The present invention further relates to a method of inhibiting NAALADase enzyme activity in an animal, comprising administering an effective amount of the compound of formula I to said animal.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a bar graph plotting the growth of the prostate cancer cell line, LNCAP, against various concentrations of quisqualic acid, a NAALADase Inhibitor. FIG. 1 shows the effect of 7-day treatment with quisqualate on the growth of LNCAP cells. Concentrations ranging from 10 nM to 1 μM, of quisqualate show a sharp dose-dependent decrease of LNCAP cell

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proliferation as indicated by the significant decrease in the incorporation of [3H]thymidine.

FIG. 2 is a bar graph plotting the growth of the prostate cancer cell line, LNCAP, against various concentrations of 2-(phosphonomethyl)pentanedioic acid, a NAALADase Inhibitor.

FIG. 2 shows the effect of 7-day treatment with 2-(phosphonomethyl)pentanedioic acid on the growth of LNCAP cells. Concentrations ranging from 100 pM to 10 nM of 2-(phosphonomethyl)pentanedioic acid show a sharp dose-dependent decrease of LNCAP cell proliferation as indicated by the significant decrease in the incorporation of [3H]thymidine.

FIG. 3 is a line graph of the response of LNCAP human prostate tumors to daily treatment with 2-(phosphonomethyl)pentanedioic acid. Mean of individual tumor volumes are plotted as a function of time after the start of treatment. Error bars represent the SEM. Treatment with 2-(phosphonomethyl)pentanedioic acid for six weeks resulted in statistically significant difference between both the control group and animals given daily injections of drug ($p=0.04$), and the control group and animals implanted with polymer ($p=0.02$).

FIG. 4 is a line graph plotting the survival percentage of animals treated with injections against the number of days. FIG. 4 shows the higher mean survival percentage of animals injected with 2-(phosphonomethyl)pentanedioic acid mixed with polymer as compared to those animals only receiving intratumoral injections of 2-(phosphonomethyl)pentanedioic acid or a vehicle control. The graph shows that 88% of the animals treated with polymer were alive after 72 days, and

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those animals had small tumors.

FIG. 5 is a line graph plotting tumor growth against days following rat dunning cell injections. Cells were injected, over a period of 84 days, with various dosages of 2-(phosphonomethyl)pentanedioic acid and a control vehicle.

FIG. 5 shows that tumor growth slowed as a function of 2-(phosphonomethyl)pentanedioic acid dosage.

FIG. 6 is a line graph of the response of R3327 rat prostate tumors to daily treatment with 2-

10 [[(phenylmethyl)hydroxyphosphinyl]methyl]pentanedioic acid.

Mean of individual tumor volumes expressed relative to the volume at the start of treatment (V/V_0) are plotted as a function of time. Treatment with 2-

15 [[(phenylmethyl)hydroxyphosphinyl]methyl]pentanedioic acid for 2.5 weeks resulted in a statistically significant difference between the control group and animals given daily injections of 1 μg of drug ($p=0.02$).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

20 "Compound 3" refers to 2-(phosphonomethyl)pentanedioic acid, a NAALADase inhibitor.

"Inhibition", in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition. Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on the reaction kinetics of an enzyme. Competitive inhibition occurs when the

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inhibitor combines reversibly with the enzyme in such a way that it competes with a normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i , which is defined as:

$$K_i = \frac{[E][I]}{[EI]}$$

wherein [E] is the concentration of the enzyme, [I] is the concentration of the inhibitor, and [EI] is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and NAALADase. "IC50" is a related term used to define the concentration or amount of a compound which is required to cause a 50% inhibition of the target enzyme.

The term "inhibition", in the context of tumor growth or tumor cell growth, may be assessed by delayed appearance of primary or secondary tumors, slowed development of primary or secondary tumors, decreased occurrence of primary or secondary tumors, slowed or decreased severity of secondary effects of disease, arrested tumor growth and regression of tumors, among others. In the extreme, complete inhibition, is referred to herein as prevention.

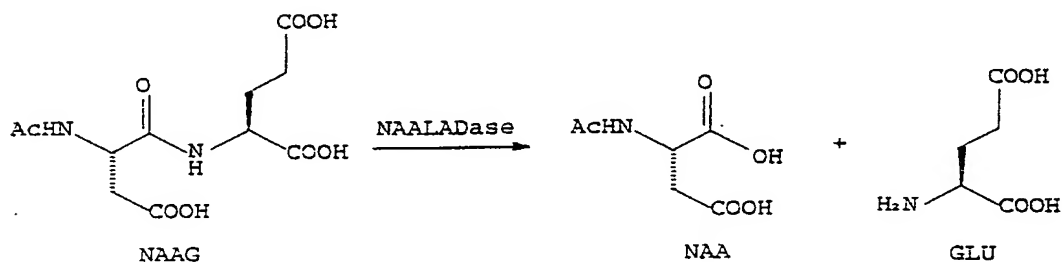
"NAAG" refers to N-acetyl-aspartyl-glutamate, an important peptide component of the brain, with levels comparable to the major inhibitor neurotransmitter gamma-aminobutyric acid (GABA). NAAG is neuron-specific, present in

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synaptic vesicles and released upon neuronal stimulation in several systems presumed to be glutamatergic. Studies suggest that NAAG may function as a neurotransmitter and/or neuromodulator in the central nervous system, or as a precursor of the neurotransmitter glutamate.

"NAALADase" refers to N-acetylated α -linked acidic dipeptidase, a membrane-bound metalloproteinase which catabolizes NAAG to N-acetylaspartate (NAA) and glutamate:

Catabolism of NAAG by NAALADase



NAALADase shows a high affinity for NAAG with a K_m of 540 nM. If NAAG is a bioactive peptide, then NAALADase may serve to inactivate NAAG'S synaptic action. Alternatively, if NAAG functions as a precursor for glutamate, the primary function of NAALADase may be to regulate synaptic glutamate availability.

"Pharmaceutically acceptable salt" refers to a salt of the inventive compounds which possesses the desired pharmacological activity and which is neither biologically nor otherwise undesirable. The salt can be formed with inorganic acids such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate,

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camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate heptanoate, hexanoate, hydrochloride hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

The term "prevention", in relation to tumor growth or tumor cell growth, means no tumor or tumor cell growth if none had occurred, no further tumor or tumor cell growth if there had already been growth.

The term "prostate disease" relates to prostate cancer such as adenocarcinoma or metastatic cancers, conditions characterized by abnormal growth of prostatic epithelial cells such as benign prostatic hyperplasia, and other conditions requiring treatment by the compounds of the present invention.

"PSA" refers to Prostate Specific Antigen, a well known

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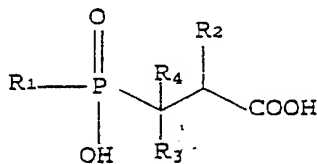
prostate cancer marker. It is a protein produced by prostate cells and is frequently present at elevated levels in the blood of men with prostate cancer. PSA correlates with tumor burden, serves as an indicator of metastatic involvement, and provides a parameter for following a prostate cancer patient's response to surgery, irradiation and androgen replacement therapy.

"PSMA" refers to Prostate Specific Membrane Antigen, a potential prostate carcinoma marker that has been hypothesized to serve as a target for imaging and cytotoxic treatment modalities for prostate cancer. PSMA is expressed in prostatic ductal epithelium and is present in seminal plasma, prostatic fluid and urine. It has been found that the expression of PSMA cDNA confers the activity of NAALADase.

The term "treatment" refers to any process, action, application, therapy, or the like, wherein an animal, including a human being, is subject to medical aid with the object of improving the animal's condition, directly or indirectly.

Preferred NAALADase Inhibitors of the Present Invention

The present invention relates to a compound of formula I:



I

or a pharmaceutically acceptable salt, hydrate, or a mixture

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thereof, wherein:

R₁ is hydrogen, C₁-C₈ straight or branched chain alkyl, C₂-C₈ straight or branched chain alkenyl group, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, or Ar₁;

5 R₂ is C₁-C₈ straight or branched chain alkyl, C₂-C₈ straight or branched chain alkenyl group, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, or Ar₁, wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl or aryl group may be optionally substituted with carboxylic
10 acid;

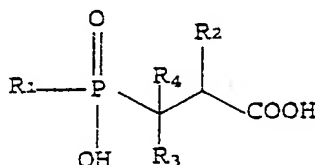
R₃ and R₄ are independently hydrogen, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, dialkyl, halogen, or Ar₁, provided that both R₃ and R₄ are not hydrogen.

15 The present invention also contemplates that said alkyl, alkenyl, cycloalkyl, cycloalkenyl or aryl groups other than R₃ and R₄ may be optionally substituted with C₁-C₈ cycloalkyl, C₁ or C₅ cycloalkyl, C₅-C₇ cycloalkenyl, C₁-C₄ alkyl, C₁-C₄ alkenyl, halo, hydroxy, carboxy, nitro, trifluoromethyl, C₁-C₆ straight
20 or branched chain alkyl or alkenyl, C₁-C₄ alkoxy, C₁-C₄ alkenyloxy, phenoxy, benzyloxy, or Ar₁, and where Ar₁ is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, 2-thienyl, 3-thienyl, 4-thienyl, 2-, 3-, or
25 4-pyridyl, or phenyl, having one to five substituents which are independently selected from the group consisting of hydrogen, halo, hydroxy, carboxy, nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl or alkenyl, C₁-C₄ alkoxy or C₁-C₄

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alkenyloxy, phenoxy, and benzyloxy; or pharmaceutically acceptable salts, hydrates, or mixtures thereof.

In a preferred embodiment, the R_1 and R_2 groups are either straight or branched aliphatic substituents or carbocyclic substituents illustrated by the compounds selected from the group of formula I:



II

wherein

- 10 R_1 is hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl group, C_3 - C_6 cycloalkyl, C_5 - C_6 cycloalkenyl, 1-naphthyl, 2-naphthyl, or phenyl;
- 15 R_2 is C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl group, C_3 - C_6 cycloalkyl, C_5 - C_6 cycloalkenyl, 1-naphthyl, 2-naphthyl, or phenyl, wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl, 1-naphthyl, 2-naphthyl, or phenyl group may be optionally substituted with
- 20 carboxylic acid;
- R_3 and R_4 are independently hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, dialkyl, halogen, or Ar_1 , provided that both R_3 and R_4 are not hydrogen.
- 25 Especially preferred methods utilize compounds wherein R_1

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is either a straight or branched aliphatic group or a carbocyclic group, and R₂ is ethyl which is substituted with a carboxylic acid are selected from the group consisting of:

2-[1-[methylhydroxyphosphinyl]ethyl]pentanedioic acid;

5 2-[1-[ethylhydroxyphosphinyl]propyl]pentanedioic acid;

2-[1-[propylhydroxyphosphinyl]butyl]pentanedioic acid;

2-[1-[butylhydroxyphosphinyl]but-2-enyl]pentanedioic acid;

2-[1-[cyclohexylhydroxyphosphinyl]pentyl]pentanedioic acid;

10 2-[1-[(cyclohexyl)methylhydroxyphosphinyl]hexyl]pentanedioic acid;

2-[1-[phenylhydroxyphosphinyl]heptyl]pentanedioic acid;

2-[1-[phenylhydroxyphosphinyl]-1-fluoromethyl]pentanedioic acid;

2-[2-[benzylhydroxyphosphinyl]propyl]pentanedioic acid;

15 2-[1-[benzylhydroxyphosphinyl]-1-phenylmethyl]pentanedioic acid;

2-[1-[phenylethylhydroxyphosphinyl]ethyl]pentanedioic acid;

2-[1-[phenylpropylhydroxyphosphinyl]propyl]pentanedioic acid;

2-[1-[phenylbutylhydroxyphosphinyl]butyl]pentanedioic acid;

20 2-[1-[(4-methylbenzyl)hydroxyphosphinyl]but-3-enyl]pentanedioic acid;

2-[1-[(4-fluorobenzyl)hydroxyphosphinyl]pentyl]pentanedioic acid;

25 2-[1-[(2-fluorobenzyl)hydroxyphosphinyl]hexyl]pentanedioic acid;

2-[1-[(pentafluorobenzyl)hydroxyphosphinyl]heptyl]pentanedioic acid;

2-[2-[(methoxybenzyl)hydroxyphosphinyl]butyl]pentanedioic

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acid;

2-[[[(4-fluorophenyl)hydroxyphosphinyl]ethyl]pentanedioic acid;

2-[1-[(hydroxy)phenylmethyl]hydroxyphosphinyl]propyl]

pentanedioic acid;

5 2-[1-[(3-methylbenzyl)hydroxyphosphinyl]butyl]pentanedioic
acid;

2-(1-phosphonobut-2-enyl)pentanedioic acid;

2-[1-[(3-trifluoromethylbenzyl)hydroxyphosphinyl]pentyl]
pentanedioic acid;

10 2-[1-[(2, 3, 4-trimethoxyphenyl)hydroxyphosphinyl]hexyl]
pentanedioic acid;

2-[1-[(1-naphthyl)hydroxyphosphinyl]heptyl]pentanedioic acid;

2-[1-[(1-naphthyl)hydroxyphosphinyl]-1-fluoromethyl]
pentanedioic acid;

15 2-[2-[(2-naphthyl)hydroxyphosphinyl]butyl]pentanedioic acid;
2-[1-[(2-naphthyl)hydroxyphosphinyl]-1-phenylmethyl]
pentanedioic acid;

2-[1-[(1-naphthyl)methylhydroxyphosphinyl]ethyl]pentanedioic
acid;

20 2-[1-[(2-naphthyl)methylhydroxyphosphinyl]propyl]pentanedioic
acid;

2-[1-[(1-naphthyl)ethylhydroxyphosphinyl]butyl]pentanedioic
acid;

2-[1-[(2-naphthyl)ethylhydroxyphosphinyl]but-3-enyl]

25 pentanedioic acid;

2-[1-[(1-naphthyl)propylhydroxyphosphinyl]pentyl]pentanedioic
acid;

2-[1-[(2-naphthyl)propylhydroxyphosphinyl]hexyl]pentanedioic

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acid;

2-[1-[(1-naphthyl)butylhydroxyphosphinyl]heptyl]pentanedioic acid;

2-[2-[(2-naphthyl)butylhydroxyphosphinyl]pentyl]pentanedioic acid; and

2-[1-[(phenylprop-2-enyl)hydroxyphosphinyl]ethyl]pentanedioic acid.

Especially preferred methods utilize compounds wherein R₁ is either a straight or branched aliphatic group or a carbocyclic group, and R₂ is ethyl which is substituted with a carboxylic acid are selected from the group consisting of:

2-[1-(benzylhydroxyphosphinyl)propyl]pentanedioic acid;

2-[1-(phenylhydroxyphosphinyl)butyl]pentanedioic acid;

2-[1-[(hydroxy)phenylmethyl]hydroxyphosphinyl]but-2-enyl]pentanedioic acid;

2-[1-(butylhydroxyphosphinyl)pentyl]pentanedioic acid;

2-[1-[(3-methylbenzyl)hydroxyphosphinyl]hexyl]pentanedioic acid;

2-[1-(3-phenylpropylhydroxyphosphinyl)heptyl]pentanedioic acid;

2-[1-(3-phenylpropylhydroxyphosphinyl)-1-fluoromethyl]pentanedioic acid;

2-[2-[(4-fluorophenyl)hydroxyphosphinyl]pent-3-enyl]pentanedioic acid;

2-[1-[(4-fluorophenyl)hydroxyphosphinyl]-1-phenylmethyl]pentanedioic acid;

2-[1-(methylhydroxyphosphinyl)ethyl]pentanedioic acid;

2-[1-(phenylethylhydroxyphosphinyl)propyl]pentanedioic acid;

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2-[1-[(4-methylbenzyl)hydroxyphosphinyl]butyl]pentanedioic acid;

2-[1-[(4-fluorobenzyl)hydroxyphosphinyl]but-3-enyl] pentanedioic acid;

5 2-[1-[(4-methoxybenzyl)hydroxyphosphinyl]pentyl]pentanedioic acid;

2-[1-[(2-fluorobenzyl)hydroxyphosphinyl]hexyl]pentanedioic acid;

10 2-[1-[(pentafluorobenzyl)hydroxyphosphinyl]heptyl]pentanedioic acid;

2-(2-phosphonopent-4-enyl)pentanedioic acid; and

2-[1-[(3-trifluoromethylbenzyl)hydroxyphosphinyl]ethyl] pentanedioic acid.

Especially preferred methods utilize compounds wherein R₁ is a straight or branched aliphatic group or a carbocyclic group, and R₂ is phenyl are selected from the group consisting of:

3-(methylhydroxyphosphinyl)-3-ethyl-2-phenylpropanoic acid;

3-(ethylhydroxyphosphinyl)-3-propyl-2-phenylpropanoic acid;

20 3-(propylhydroxyphosphinyl)-3-prop-2-enyl-2-phenylpropanoic acid;

3-(butylhydroxyphosphinyl)-3-t-butyl-2-phenylpropanoic acid;

3-(cyclohexylhydroxyphosphinyl)-3-pentyl-2-phenylpropanoic acid;

25 3-((cyclohexyl)methylhydroxyphosphinyl)-3-hexyl-2-phenyl propanoic acid;

3-((cyclohexyl)methylhydroxyphosphinyl)-3-fluoro-2-phenyl propanoic acid;

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3-(phenylhydroxyphosphinyl)-3-methyl-3-butyl-2-phenylpropanoic acid;

3-(phenylhydroxyphosphinyl)-2,3-diphenylpropanoic acid; . .

3-(benzylhydroxyphosphinyl)-3-methyl-2-phenylpropanoic acid;

5 3-(phenylethylhydroxyphosphinyl)-3-ethyl-2-phenylpropanoic acid;

3-(phenylpropylhydroxyphosphinyl)-3-propyl-2-phenylpropanoic acid;

10 3-(phenylbutylhydroxyphosphinyl)-3-prop-1-enyl-2-phenylpropanoic acid;

3-[(2, 3, 4-trimethoxyphenyl)-3-hydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic acid;

3-[(1-naphthyl)hydroxyphosphinyl]-3-pentyl-2-phenylpropanoic acid;

15 3-[(2-naphthyl)hydroxyphosphinyl]-3-hexyl-2-phenylpropanoic acid;

3-[(1-naphthyl)methylhydroxyphosphinyl]-3-methyl-3-pentyl-2-phenylpropanoic acid;

20 3-[(2-naphthyl)methylhydroxyphosphinyl]-3-methyl-2-phenylpropanoic acid;

3-[(1-naphthyl)ethylhydroxyphosphinyl]-3-ethyl-2-phenylpropanoic acid;

3-[(2-naphthyl)ethylhydroxyphosphinyl]-3-propyl-2-phenylpropanoic acid;

25 3-[(1-naphthyl)propylhydroxyphosphinyl]-3-prop-2-enyl-2-phenylpropanoic acid;

3-[(2-naphthyl)propylhydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic acid;

-19-

3-[(1-naphthyl)butylhydroxyphosphinyl]-3-pentyl-2-phenyl
propanoic acid;

3-[(2-naphthyl)butylhydroxyphosphinyl]-3-hexyl-2-phenyl
propanoic acid; and

5 3-[phenylprop-2-enylhydroxyphosphinyl]-3-methyl-3-hexyl-2-
phenylpropanoic acid.

Although not limited to any one species, a highly
preferred species where R_1 is a straight or branched aliphatic
group or a carbocyclic group and R_2 is ethyl which is
10 substituted with carboxylic acid is

2-[1-[benzylhydroxyphosphinyl]ethyl]pentanedioic acid.

Other preferred compounds of the present invention are
selected from the group consisting of:

hydroxyphosphinyl derivatives wherein, R_1 is a straight or
15 branched aliphatic group or a carbocyclic group and R_2 is an
 C_2 - C_8 alkyl or alkenyl chain which is substituted with a
carboxylic acid. Exemplary species include:

2-[1-(methylhydroxyphosphinyl)propyl]hexanedioic acid;

2-[1-(benzylhydroxyphosphinyl)butyl]hexanedioic acid;

20 2-[1-(methylhydroxyphosphinyl)but-2-enyl]heptanedioic acid;

2-[1-(benzylhydroxyphosphinyl)pentyl]heptanedioic acid;

2-[1-(methylhydroxyphosphinyl)hexyl]octanedioic acid;

2-[1-(benzylhydroxyphosphinyl)heptyl]octanedioic acid;

2-[1-(benzylhydroxyphosphinyl)-1-fluoromethyl]octanedioic
25 acid;

2-[3-(methylhydroxyphosphinyl)pentyl]nonanedioic acid;

2-[1-(methylhydroxyphosphinyl)-1-phenylmethyl]nonanedioic
acid;

-20-

2-[1-(benzylhydroxyphosphinyl)ethyl]nonanedioic acid;
2-[1-(methylhydroxyphosphinyl)propyl]decanedioic acid; and
2-[1-(benzylhydroxyphosphinyl)butyl]decanedioic acid.

5 Other preferred compounds are selected from the group
consisting of:

hydroxyphosphinyl derivatives wherein R₁ is benzyl and R₂ is a
straight or branched aliphatic group or a carbocyclic group.

Exemplary species include:

- 10 3-(benzylhydroxyphosphinyl)-3-prop-2-enyl-2-methylpropanoic
acid;
3-(benzylhydroxyphosphinyl)-3-t-butyl-2-ethylpropanoic acid;
3-(benzylhydroxyphosphinyl)-3-pentyl-2-propylpropanoic acid;
3-(benzylhydroxyphosphinyl)-3-hexyl-2-butylpropanoic acid;
15 3-(benzylhydroxyphosphinyl)-3-fluoro-2-butylpropanoic acid;
3-(benzylhydroxyphosphinyl)-3-ethyl-3-propyl-2-cyclohexyl
propanoic acid;
3-(benzylhydroxyphosphinyl)-3-phenyl-2-cyclohexylpropanoic
acid;
20 3-(benzylhydroxyphosphinyl)-3-methyl-2-(cyclohexyl)methyl
propanoic acid;
3-(benzylhydroxyphosphinyl)-3-ethyl-2-phenylpropanoic acid;
3-(benzylhydroxyphosphinyl)-3-propyl-2-benzylpropanoic acid;
3-(benzylhydroxyphosphinyl)-3-prop-1-enyl-2-phenylethyl
25 propanoic acid;
3-(benzylhydroxyphosphinyl)-3-t-butyl-2-phenylpropylpropanoic
acid;
3-(benzylhydroxyphosphinyl)-3-pentyl-2-phenylbutylpropanoic

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acid;

3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2, 3, 4-trimethoxyphenyl) propanoic acid;

3-(benzylhydroxyphosphinyl)-3-ethyl-3-prop-1-enyl-2-(1-naphthyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-methyl-2-(2-naphthyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-ethyl-2-(1-naphthyl)methyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-propyl-2-(2-naphthyl)methyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-prop-2-enyl-2-(1-naphthyl)ethyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(2-naphthyl)ethyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-pentyl-2-(1-naphthyl)propyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2-naphthyl)propyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-fluoro-2-(2-naphthyl)propyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-ethyl-3-butyl-2-(1-naphthyl)butylpropanoic acid;

3-(benzylhydroxyphosphinyl)-3-phenyl-2-(1-naphthyl)butyl propanoic acid;

3-(benzylhydroxyphosphinyl)-3-methyl-2-(2-naphthyl)butyl propanoic acid; and

3-(benzylhydroxyphosphinyl)-3-ethyl-2-phenylprop-2-enyl

-22-

propanoic acid.

Especially preferred methods use compounds wherein R_1 is said alkyl, alkenyl, cycloalkyl, or aryl group which is substituted with a heterocyclic group and R_2 is ethyl which is substituted with a carboxylic acid are selected from the group consisting of:

- 2-[1-[(2-pyridyl)methylhydroxyphosphinyl]butyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)methylhydroxyphosphinyl]but-2-enyl]pentanedioic acid;
- 2-[1-[(4-pyridyl)methylhydroxyphosphinyl]pentyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)ethylhydroxyphosphinyl]hexyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)propylhydroxyphosphinyl]heptyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)propylhydroxyphosphinyl]-1-fluoromethyl]pentanedioic acid;
- 2-[3-[(tetrahydrofuranyl)methylhydroxyphosphinyl]octyl]pentanedioic acid;
- 2-[1-[(tetrahydrofuranyl)methylhydroxyphosphinyl]-1-phenylmethyl]pentanedioic acid;
- 2-[1-[(tetrahydrofuranyl)ethylhydroxyphosphinyl]ethyl]pentanedioic acid;
- 2-[1-[(tetrahydrofuranyl)propylhydroxyphosphinyl]propyl]pentanedioic acid;
- 2-[1-[(2-indolyl)methylhydroxyphosphinyl]butyl]pentanedioic acid;

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2-[1-[(3-indolyl)methylhydroxyphosphinyl]but-3-enyl]

pentanedioic acid;

2-[1-[(4-indolyl)methylhydroxyphosphinyl]pentyl]pentanedioic acid;

5 2-[1-[(3-indolyl)ethylhydroxyphosphinyl]hexyl]pentanedioic acid;

2-[1-[(3-indolyl)propylhydroxyphosphinyl]heptyl]pentanedioic acid;

10 2-[3-[(2-thienyl)methylhydroxyphosphinyl]nonyl]pentanedioic acid;

2-[1-[(3-thienyl)methylhydroxyphosphinyl]ethyl]pentanedioic acid;

2-[1-[(4-thienyl)methylhydroxyphosphinyl]propyl]pentanedioic acid;

15 2-[1-[(3-thienyl)ethylhydroxyphosphinyl]butyl]pentanedioic acid; and

2-[1-[(3-thienyl)propylhydroxyphosphinyl]but-2-enyl]pentanedioic acid.

Especially preferred methods use compounds wherein R₁ is said alkyl, alkenyl, cycloalkyl, or aryl group which is substituted with a heterocyclic group and R₂ is phenyl are selected from the group consisting of:

3-[(2-pyridyl)methylhydroxyphosphinyl]-3-t-butyl-2-phenyl propanoic acid;

25 3-[(3-pyridyl)methylhydroxyphosphinyl]-3-pentyl-2-phenyl propanoic acid;

3-[(4-pyridyl)methylhydroxyphosphinyl]-3-hexyl-2-phenyl propanoic acid;

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- 3-[(4-pyridyl)methylhydroxyphosphinyl]-3-fluoro-2-phenyl
propanoic acid;
- 3-[(3-pyridyl)ethylhydroxyphosphinyl]-3-dipropyl-2-phenyl
propanoic acid;
- 5 3-[(3-pyridyl)ethylhydroxyphosphinyl]-2,3-diphenylpropanoic
acid;
- 3-[(3-pyridyl)propylhydroxyphosphinyl]-3-methyl-2-phenyl
propanoic acid;
- 3-[(tetrahydrofuranyl)methylhydroxyphosphinyl]-3-ethyl-2-
10 phenylpropanoic acid;
- 3-[(tetrahydrofuranyl)ethylhydroxyphosphinyl]-3-propyl-2-
phenylpropanoic acid;
- 3-[(tetrahydrofuranyl)propylhydroxyphosphinyl]-3-prop-2-enyl-
2-phenylpropanoic acid;
- 15 3-[(2-indolyl)methylhydroxyphosphinyl]-3-t-butyl-2-phenyl
propanoic acid;
- 3-[(3-indolyl)methylhydroxyphosphinyl]-3-pentyl-2-phenyl
propanoic acid;
- 3-[(4-indolyl)methylhydroxyphosphinyl]-3-hexyl-2-phenyl
20 propanoic acid;
- 3-[(3-indolyl)ethylhydroxyphosphinyl]-3-propyl-3-t-butyl-2-
phenylpropanoic acid;
- 3-[(3-indolyl)propylhydroxyphosphinyl]-3-methyl-2-phenyl
propanoic acid;
- 25 3-[(2-thienyl)methylhydroxyphosphinyl]-3-ethyl-2-phenyl
propanoic acid;
- 3-[(3-thienyl)methylhydroxyphosphinyl]-3-propyl-2-phenyl
propanoic acid;

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3-[(4-thienyl)methylhydroxyphosphinyl]-3-prop-1-enyl-2-phenyl
propanoic acid;

3-[(3-thienyl)ethylhydroxyphosphinyl]-3-t-butyl-2-phenyl
propanoic acid; and

5 3-[(3-thienyl)propylhydroxyphosphinyl]-3-pentyl-2-phenyl
propanoic acid.

Especially preferred methods use compounds wherein R₁ is
benzyl and R₂ is said alkyl, alkenyl, cycloalkyl, or aryl group
which is substituted with a heterocyclic group are selected

10 from the group consisting of:

3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2-pyridyl)methyl
propanoic acid;

3-(benzylhydroxyphosphinyl)-3-fluoro-2-(2-pyridyl)methyl
propanoic acid;

15 3-(benzylhydroxyphosphinyl)-3-propyl-3-pentyl-2-(3-pyridyl)
methylpropanoic acid;

3-(benzylhydroxyphosphinyl)-3-phenyl-2-(3-pyridyl)methyl
propanoic acid;

20 3-(benzylhydroxyphosphinyl)-3-methyl-2-(4-pyridyl)methyl
propanoic acid;

3-(benzylhydroxyphosphinyl)-3-ethyl-2-(3-pyridyl)
ethylpropanoic acid;

3-(benzylhydroxyphosphinyl)-3-propyl-2-(3-pyridyl)propyl
propanoic acid;

25 3-(benzylhydroxyphosphinyl)-3-prop-2-enyl-2-
(tetrahydrofuranyl)methylpropanoic acid;

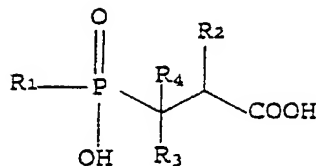
3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(tetrahydrofuranyl)
ethylpropanoic acid;

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- 3-(benzylhydroxyphosphinyl)-3-pentyl-2-(tetrahydrofuranyl)
propylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2-indolyl)methyl
propanoic acid;
- 5 3-(benzylhydroxyphosphinyl)-3-propyl-3-hexyl-2-(3-indolyl)
methylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-methyl-2-(4-indolyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-ethyl-2-(3-indolyl)ethyl
10 propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-propyl-2-(3-indolyl)propyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-prop-1-enyl-2-(2-thienyl)methyl
propanoic acid;
- 15 3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(3-thienyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-pentyl-2-(4-thienyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(3-thienyl)ethyl
20 propanoic acid; and
- 3-(benzylhydroxyphosphinyl)-3-t-butyl-3-pentyl-2-(3-thienyl)
propylpropanoic acid.

In another preferred embodiment, the R groups are
heterocyclic substituents illustrated by the compounds
25 selected from the group having formula I:

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wherein

II

R_1 is Ar_1 ;

5 R_2 is C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl group, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, or Ar_1 , wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl or aryl group may be optionally substituted with carboxylic acid;

R_3 and R_4 are independently hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, dialkyl, halogen, or Ar_1 , provided that both R_3 and R_4 are not hydrogen.

15 Especially preferred methods use compounds wherein R_1 is a heterocyclic group and R_2 is ethyl which is substituted with carboxylic acid are selected from the group consisting of:

2-[1-[(2-pyridyl)hydroxyphosphinyl]ethyl]pentanedioic acid;
 2-[1-[(3-pyridyl)hydroxyphosphinyl]propyl]pentanedioic acid;
 20 2-[1-[(4-pyridyl)hydroxyphosphinyl]butyl]pentanedioic acid;
 2-[1-[(tetrahydrofuranyl)hydroxyphosphinyl]but-3-enyl]
 pentanedioic acid;
 2-[1-[(2-indolyl)hydroxyphosphinyl]pentyl]pentanedioic acid;
 2-[1-[(3-indolyl)hydroxyphosphinyl]hexyl]pentanedioic acid;
 25 2-[1-[(4-indolyl)hydroxyphosphinyl]heptyl]pentanedioic acid;

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2-[1-[(4-indolyl)hydroxyphosphinyl]-1-fluoromethyl]

pentanedioic acid;

2-[2-[(2-thienyl)hydroxyphosphinyl]propyl]pentanedioic acid;

2-[1-[(2-thienyl)hydroxyphosphinyl]-1-phenylmethyl]

5 pentanedioic acid;

2-[1-[(3-thienyl)hydroxyphosphinyl]ethyl]pentanedioic acid;

and

2-[1-[(4-thienyl)hydroxyphosphinyl]propyl]pentanedioic acid.

Compounds of the present invention wherein R₁ is a
10 heterocyclic group and R₂ is phenyl are selected from the group
consisting of:

3-[(2-pyridyl)hydroxyphosphinyl]-3-prop-1-enyl-2-phenyl

propanoic acid;

3-[(3-pyridyl)hydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic

15 acid;

3-[(4-pyridyl)hydroxyphosphinyl]-3-pentyl-2-phenylpropanoic

acid;

3-[(tetrahydrofuranyl)hydroxyphosphinyl]-3-hexyl-2-phenyl

propanoic acid;

20 3-[(tetrahydrofuranyl)hydroxyphosphinyl]-3-fluoro-2-phenyl

propanoic acid;

3-[(2-indolyl)hydroxyphosphinyl]-3-t-butyl-3-hexyl-2-phenyl

propanoic acid;

3-[(2-indolyl)hydroxyphosphinyl]-2,3-diphenylpropanoic acid;

25 3-[(3-indolyl)hydroxyphosphinyl]-3-methyl-2-phenylpropanoic

acid;

3-[(4-indolyl)hydroxyphosphinyl]-3-ethyl-2-phenylpropanoic

acid;

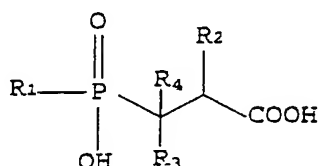
-29-

3-[(2-thienyl)hydroxyphosphinyl]-3-propyl-2-phenylpropanoic acid;

3-[(3-thienyl)hydroxyphosphinyl]-3-prop-2-enyl-2-phenylpropanoic acid; and

5 3-[(4-thienyl)hydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic acid.

Compounds are also preferably selected from the group of formula I:



10

II

wherein

R_1 is hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl group, C_3 - C_6 cycloalkyl, C_5 - C_7 cycloalkenyl, or Ar_1 ;

15

R_2 is Ar_1 , wherein said aryl group may be optionally substituted with carboxylic acid;

R_3 and R_4 are independently hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, dialkyl, halogen, or Ar_1 , provided

20

that both R_3 and R_4 are not hydrogen.

Particular species wherein R_2 is heterocyclic may be easily made and used by persons of ordinary skill in the art in accordance with the teachings provided herein and known in the art.

25

Compounds wherein R_1 is benzyl and R_2 is heterocyclic are

-30-

selected from the group consisting of:

3-(benzylhydroxyphosphinyl)-3-pentyl-2-(2-pyridyl)propanoic acid;

5 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(3-pyridyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-fluoro-2-(3-pyridyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-pentyl-3-hexyl-2-(4-pyridyl)propanoic acid;

10 3-(benzylhydroxyphosphinyl)-3-phenyl-2-(4-pyridyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-methyl-2-(tetrahydrofuranyl)propanoic acid;

15 3-(benzylhydroxyphosphinyl)-3-ethyl-2-(2-indolyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-propyl-2-(3-indolyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-prop-1-enyl-2-(4-indolyl)propanoic acid;

20 3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(2-thienyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-pentyl-2-(3-thienyl)propanoic acid; and

25 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(4-thienyl)propanoic acid.

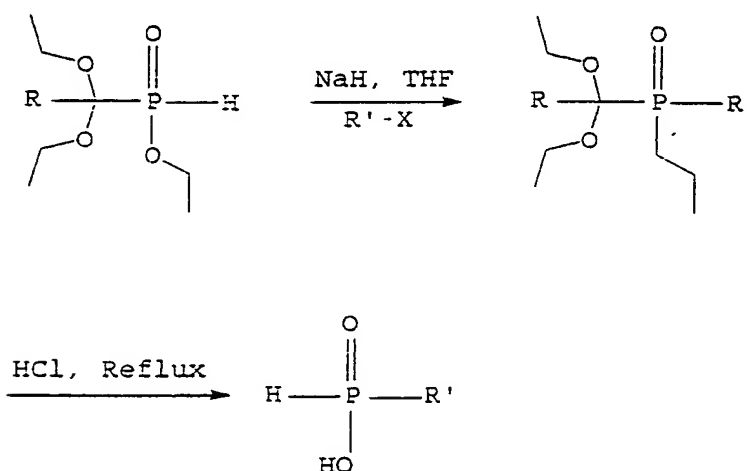
Synthesis of Compounds

The compounds of the present invention can be readily

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prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways depicted below (see Schemes I-IX). Precursor compounds may be prepared by methods known in the art, such as those described in the method of Jackson et al. (*J. Med. Chem.* 39(2), 619-622, Design, Synthesis, and Biological Activity of a Potent Inhibitor of the Neuropeptidase N-Acetylated α -Linked Acidic Dipeptidase) and, for example, in Froestl et al. (*J. Med. Chem.*, 1995, 38, 3313-3331, Phosphinic Acid Analogues of GABA).

SCHEME I

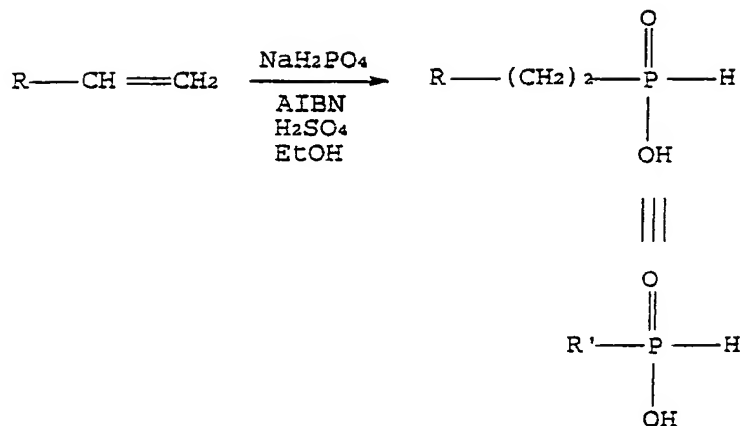


Production of compounds containing the R group substitutions can be easily made utilizing known methods. Further methods of synthesizing phosphinic acid esters are also described in *J. Med. Chem.*, 1988, 31, 204-212, and may be

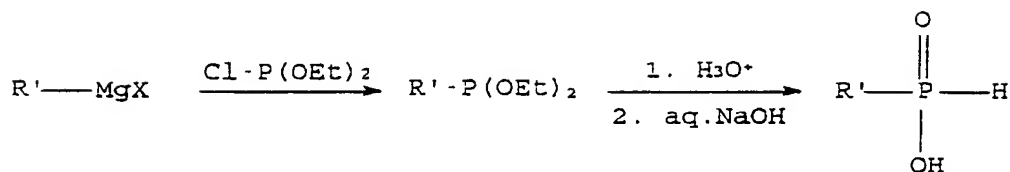
-32-

found in Scheme II, below.

SCHEME II.



- | | | | |
|----|--|----|---|
| A. | R' = (CH ₂) ₃ Ph | H. | n-C ₇ H ₁₅ |
| B. | (CH ₂) ₄ Ph | I. | n-C ₈ H ₁₇ |
| C. | (CH ₂) ₅ Ph | J. | n-C ₉ H ₁₉ |
| D. | (CH ₂) ₄ (P-F-Ph) | K. | n-C ₁₀ H ₂₁ |
| E. | (CH ₂) ₄ -(3-pyridyl) | L. | CH ₂ (CH ₂)(CH ₃)C ₄ H ₉ |
| F. | n-C ₅ H ₁₁ | M. | CH ₂ (CH ₂)CH(CH ₃) ₂ |
| G. | n-C ₅ H ₁₁ | | |

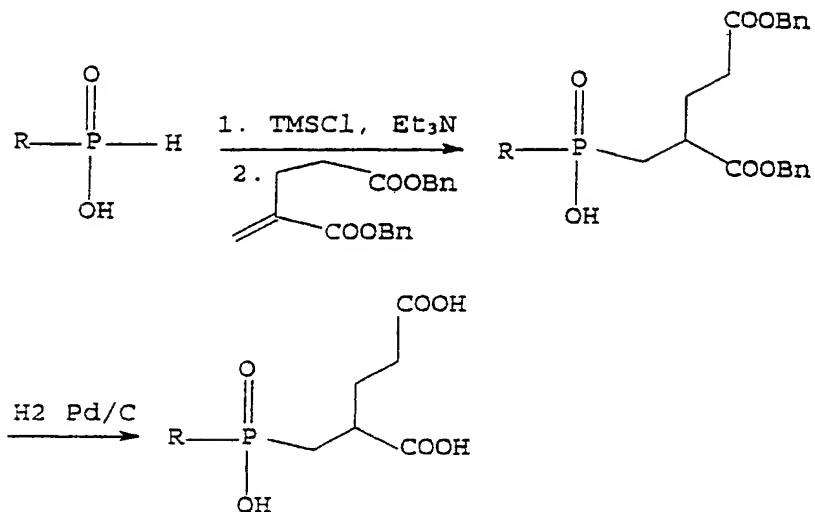


- N. R' = n-C₄H₉
 O. CH(CH₃)C₅H₁₁

Starting with the aforementioned phosphinic acid esters, there are a variety of routes that can be used to prepare the compounds of the present invention. For example, a general route was recently described in *J. Med. Chem.*, 1996, 39, 619-

-33-

622, and is set forth below in Scheme III.

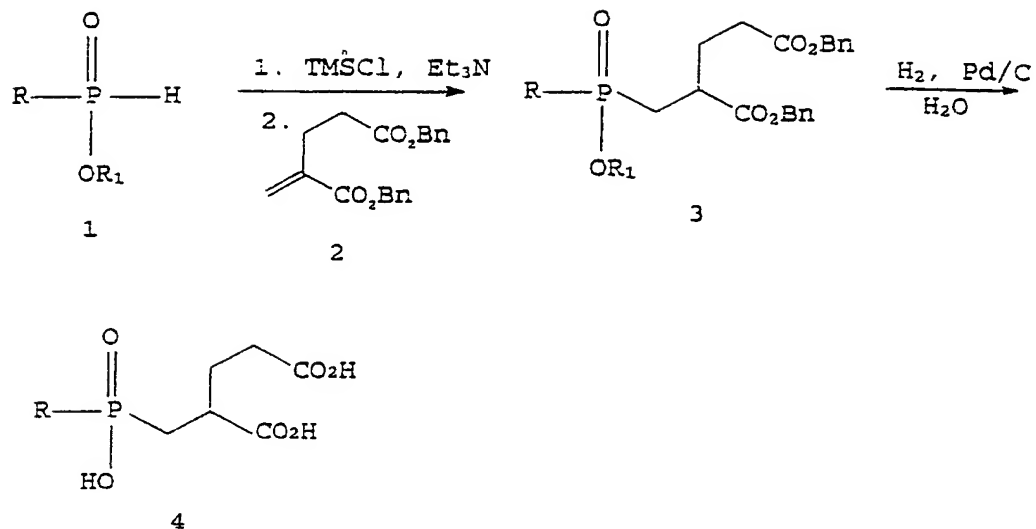


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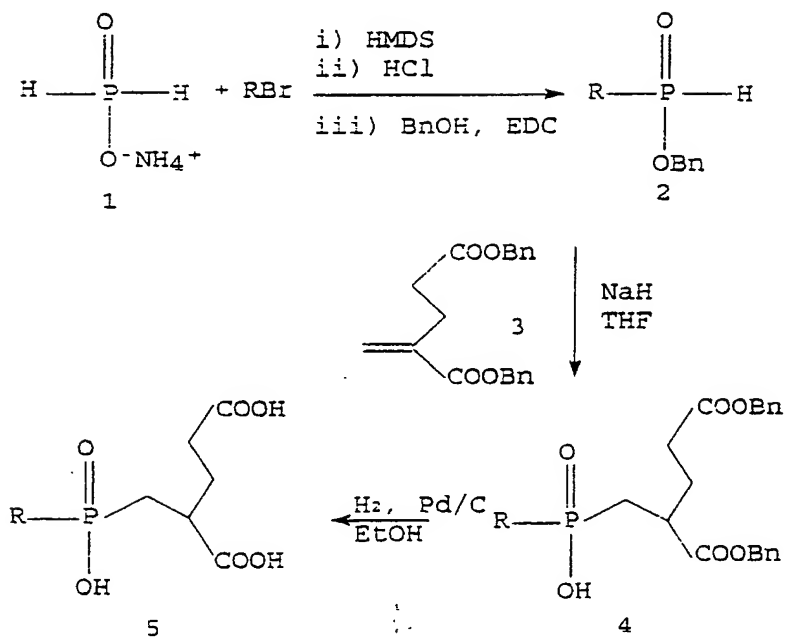
Another route for preparing the compounds of the present invention is as set forth below in scheme IV and Scheme V. Scheme IV and Scheme V also show a phosphinic acid derivative as a starting material to prepare the compounds of the present invention and the R group is contemplated as including any reasonable chemical substitution and includes without limitation the R groups listed in Scheme II and within the specification.

10

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SCHEME IV

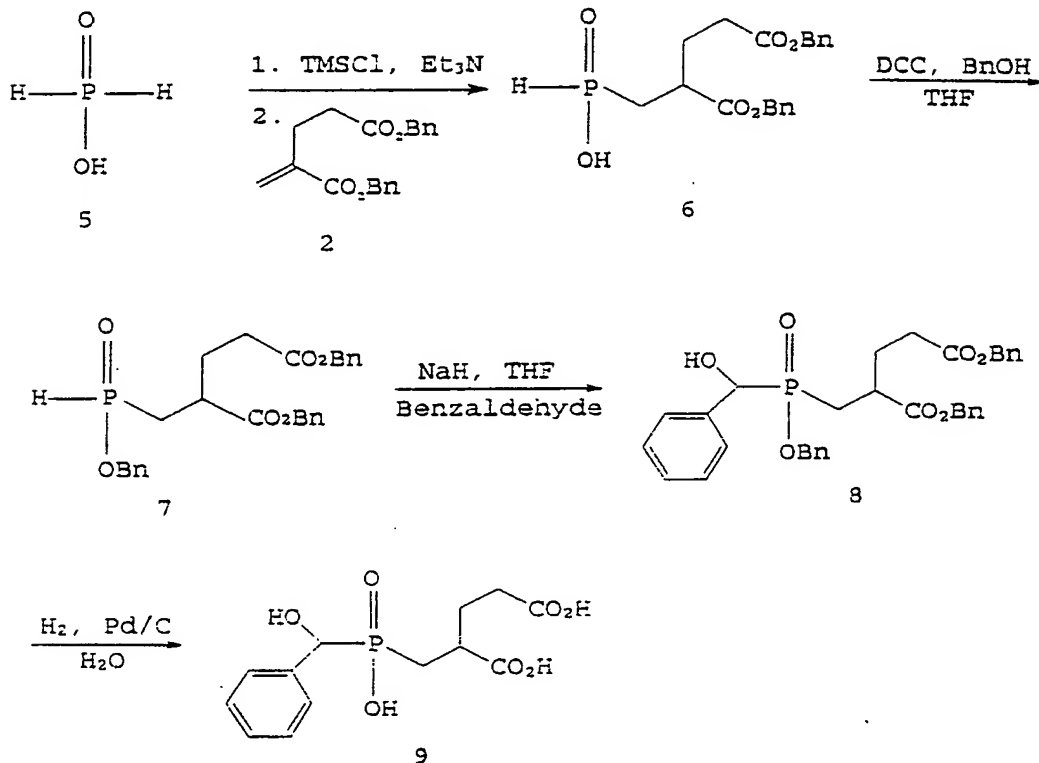


SCHEME V

-35-

Another route of preparing the compounds of the present invention allows for aromatic substitution at R1 and is set forth below in Scheme VI.

5

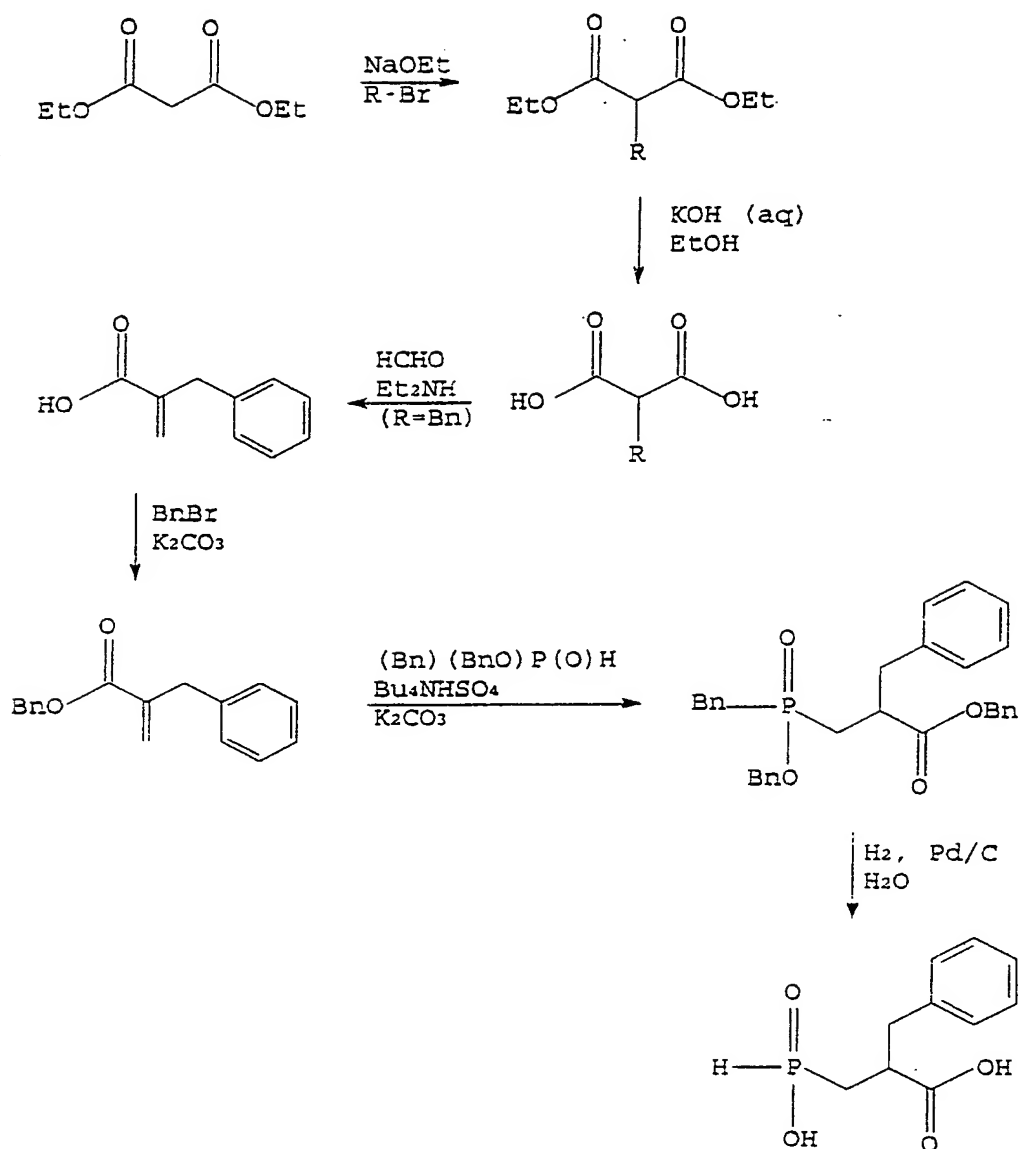


SCHEME VI

10

Another route of preparing the compounds of the present invention allows for aromatic substitution at the R2 position and is set forth below in Scheme VII.

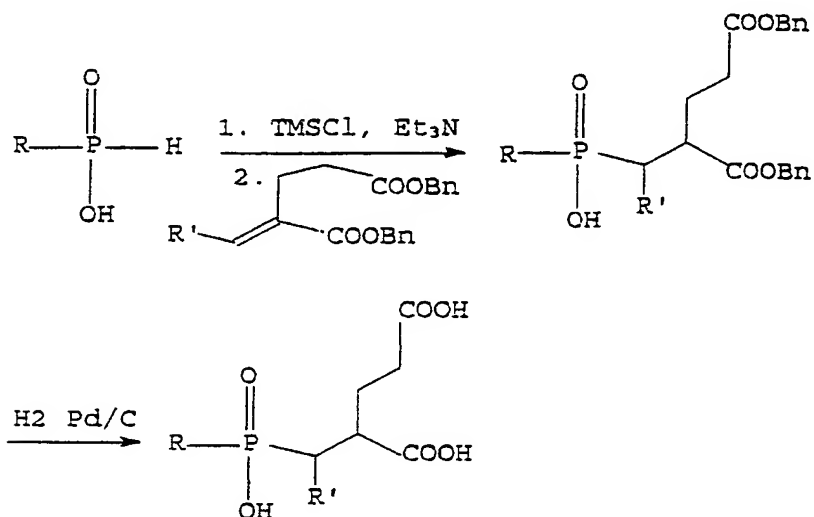
-36-



SCHEME VII

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Another route of preparing the compounds of the present invention allows for alkyl or alkenyl substitution at the R_1 and/or R_2 positions and is set forth below in Scheme VIII.



SCHEME VIII

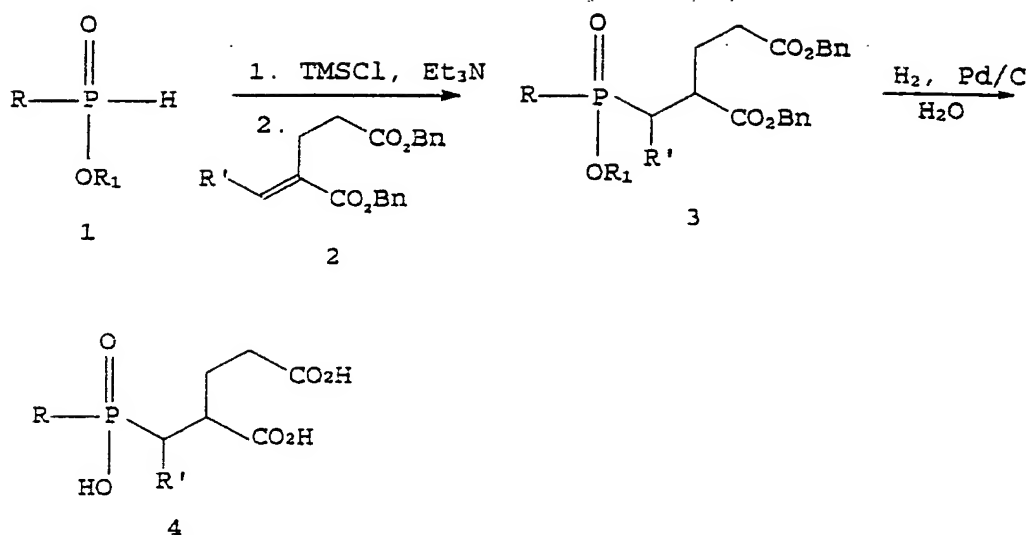
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- A. $R' = CH_3$
 B. CH_2CH_3
 C. $(CH_2)_2CH_3$
 D. $CHCHCH_3$
 10 E. CH_2CHCH_2
 F. $(CH_2)_3CH_3$
 G. $(CH_2)_4CH_3$
 H. $(CH_2)_5CH_3$
 I. Fluorine
 15 J. Phenyl

Another route for preparing the compounds of the present invention is as set forth below in Scheme IX and Scheme X. Scheme IX and Scheme X also show a phosphinic acid derivative as a starting material to prepare the compounds of the present invention and the R and R' groups are contemplated as including any reasonable chemical substitution and include without limitation the R groups listed in Scheme II and the R' groups listed in Scheme VIII and within the specification.

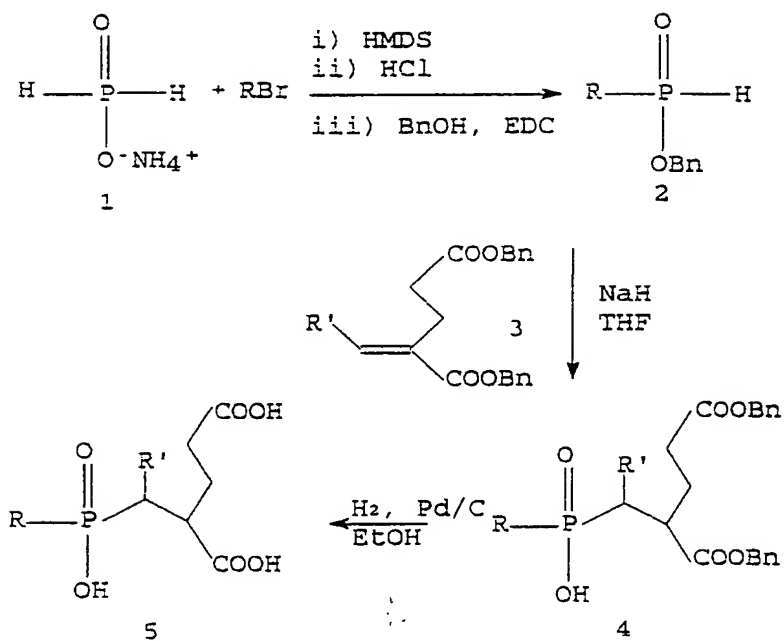
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SCHEME IX

5

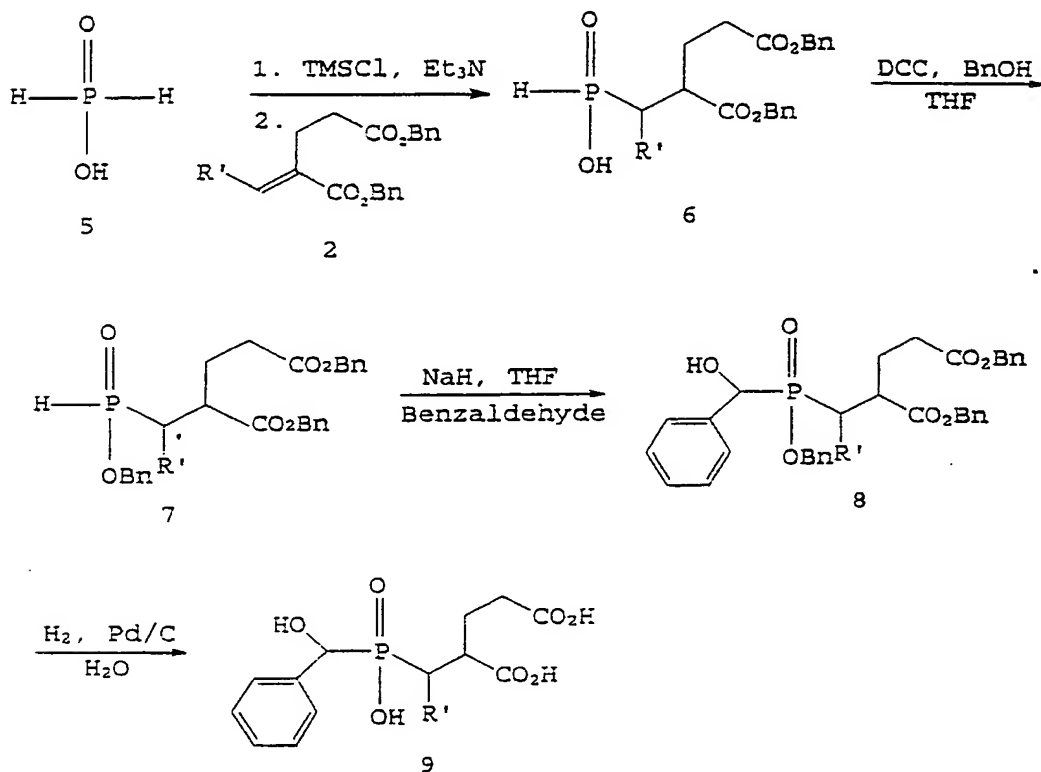


SCHEME X

-39-

Another route of preparing the compounds of the present invention allows for aromatic substitution at R_1 along with alkyl or alkenyl substitution at the R_3 and/or R_4 positions and is set forth below in Scheme XI.

5

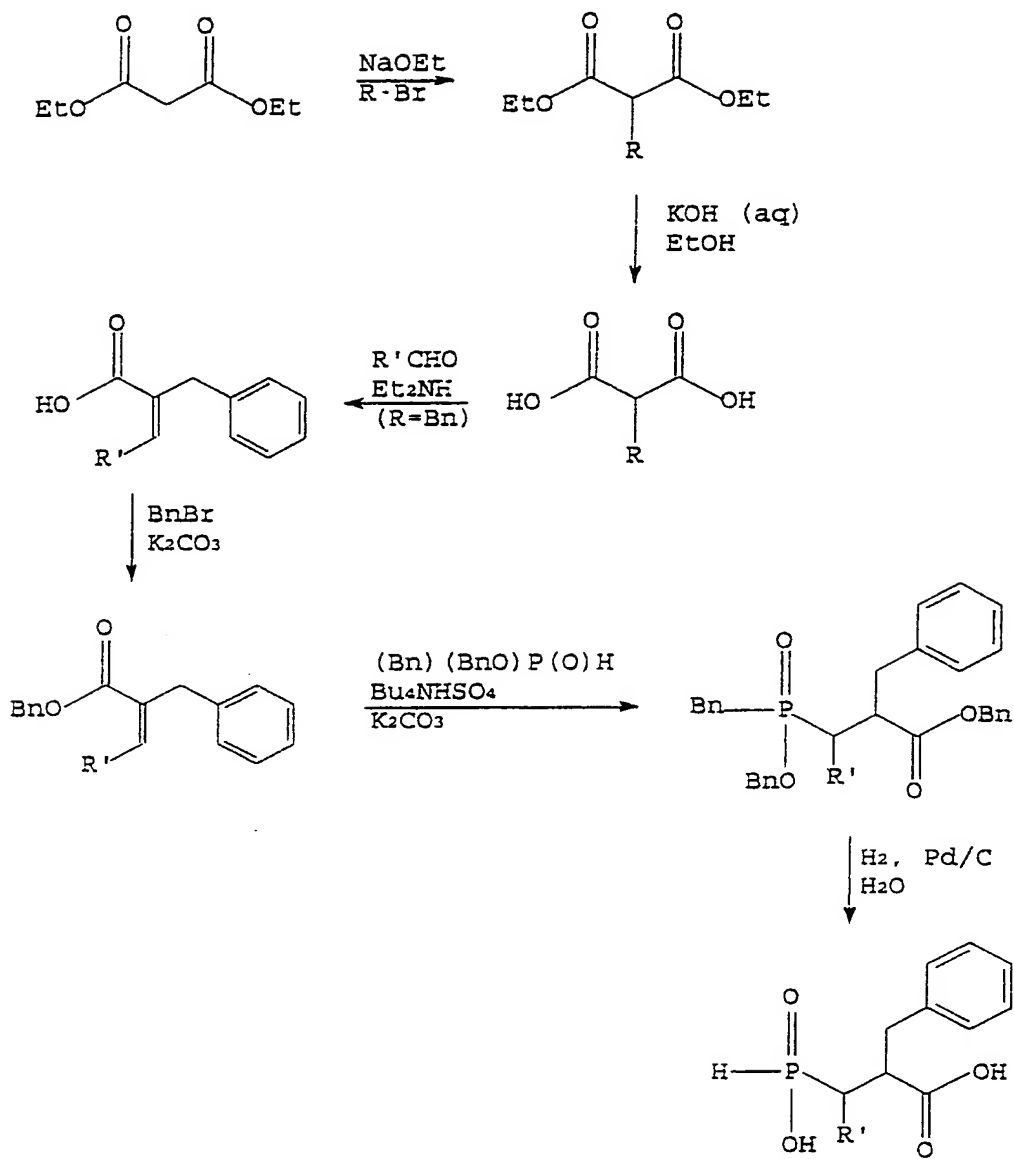


SCHEME XI

10

Another route of preparing the compounds of the present invention allows for aromatic substitution at the R_2 position along with alkyl or alkenyl substitution at the R_3 and/or R_4 positions and is set forth below in Scheme XII.

-40-



SCHEME XII

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Pharmaceutical Compositions of the Present Invention

The present invention also relates to a pharmaceutical composition comprising:

(i) a therapeutically effective amount of a compound of formula I; and

(ii) a pharmaceutically acceptable carrier.

In another preferred embodiment, the pharmaceutical composition further comprises a therapeutic agent selected from the group consisting of therapeutic hormones, chemotherapeutic agents, monoclonal antibodies, anti-angiogenesis agents, radiolabelled compounds, antineoplastic agents and mixtures thereof. Examples of therapeutic hormones include diethylstilbestrol (DES), leuprolide, flutamide, cyproterone acetate, ketoconazole and amino glutethimide are preferred. Examples of antineoplastic agents include 5-fluorouracil, vinblastine sulfate, estramustine phosphate, suramin and strontium-89. Examples of chemotherapeutic agents include buserelin, chlorotranisene, chromic phosphate, cisplatin, cyclophosphamide, dexamethasone, doxorubicin, estradiol, estradiol valerate, estrogens conjugated and esterified, estrone, ethinyl estradiol, floxuridine, goserelin, hydroxyurea, melphalan, methotrexate, mitomycin and prednisone.

In a further preferred embodiment, the compound of formula I is present in an amount that is effective for inhibiting NAALADase activity in an animal or treating a prostate disease in an animal.

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Process for Preparing Pharmaceutical Compositions

In yet another preferred embodiment, a process for preparing a pharmaceutical composition or medicament containing a compound of the present invention for treating a disease is also contemplated.

Methods of Use of the Present Inventioni) Method of inhibiting NAALADase enzyme activity

The present invention further relates to a method of inhibiting NAALADase enzyme activity in an animal, comprising administering an effective amount of a compound of formula I to said animal.

ii) Method of treating a prostate disease

The present invention also relates to a method of treating a prostate disease in an animal, comprising administering an effective amount of a compound of formula I to said animal.

In a preferred embodiment, said prostate disease is prostate cancer such as prostatic adenocarcinoma, benign prostatic hyperplasia, or conditions involving the prostate requiring administration of the compounds of the present invention, such prostatic intraepithelial neoplasia (PIN).

iii) Method of treating cancer

In addition to prostate cancer, other forms of cancer that may be treated with the compounds of the present

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invention include without limitation: ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervix cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head & neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and/or non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovary cancer, ovary (germ cell) cancer, pancreatic cancer, penis cancer, retinoblastoma, skin cancer, soft-tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, cancer of the uterus, vaginal cancer, cancer of the vulva and Wilm's tumor.

The compounds of the present invention are particularly useful in treating cancer of tissues where NAALADase enzymes reside. Such tissues include the prostate as well as the brain, kidney and testis.

For patients who initially present without advanced or metastatic cancer, NAALADase inhibitor based drugs are used as an immediate initial therapy prior to surgery and radiation therapy, and as a continuous post-treatment therapy in patients at risk for recurrence or metastasis (based upon high PSA, high Gleason's score, locally extensive disease, and/or pathological evidence of tumor invasion in the surgical

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specimen). The goal in these patients is to inhibit the growth of potentially metastatic cells from the primary tumor during surgery or radiotherapy and inhibit the growth of tumor cells from undetectable residual primary tumor.

5 For patients who initially present with advanced or metastatic cancer, NAALADase inhibitor based drugs are used as a continuous supplement to, or possible as a replacement for hormonal ablation. The goal in these patients is to slow tumor cell growth from both the untreated primary tumor and
10 from the existing metastatic lesions.

In addition, the invention may be particularly efficacious during post-surgical recovery, where the present compositions and methods may be particularly effective in lessening the chances of recurrence of a tumor engendered by
15 shed cells that cannot be removed by surgical intervention.

iv) Diagnostic kits

The present invention also includes a diagnostic kit for performing the methods of the present invention and may
20 contain compounds and/or compositions containing the compounds of the present invention. Radiolabelled compounds and monoclonal antibodies may be used in a manner so as to provide diagnostic information. Examples of diagnostic information and uses include determining the type of disease, the progress
25 of the particular disease, the location of cells targeted by a NAALADase inhibitor, radiolabelled compound or monoclonal antibody, and similar diagnostic uses known to persons skilled in the art.

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Route of Administration

In the methods of the present invention, the compounds may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an
5 implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal
10 or intracranial injection and infusion techniques. Invasive techniques are preferred, particularly direct administration to damaged neuronal tissue.

To be effective therapeutically as central nervous system targets, the compounds of the present invention should readily
15 penetrate the blood-brain barrier when peripherally administered. Compounds which cannot penetrate the blood-brain barrier can be effectively administered by an intraventricular route.

The compounds may also be administered in the form of
20 sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions. These suspensions can be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may
25 also be sterile injectable solutions or suspensions in non-toxic parenterally-acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's

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solution and isotonic sodium chloride solution. In addition, sterile fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil such as a synthetic mono- or di-glyceride may be employed.

5 Fatty acids such as oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated forms, are useful in the preparation of injectables. These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

10 Additionally, the compounds may be administered orally in the form of capsules, tablets, aqueous suspensions or solutions. Tablets may contain carriers such as lactose and corn starch, and/or lubricating agents such as magnesium stearate. Capsules may contain diluents including lactose and
15 dried corn starch. Aqueous suspensions may contain emulsifying and suspending agents combined with the active ingredient. The oral dosage forms may further contain sweetening and/or flavoring and/or coloring agents.

The compounds may further be administered rectally in the
20 form of suppositories. These compositions can be prepared by mixing the drug with suitable non-irritating excipients which are solid at room temperature, but liquid at rectal temperature such that they will melt in the rectum to release the drug. Such excipients include cocoa butter, beeswax and
25 polyethylene glycols.

Moreover, the compounds may be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application,

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including neurological disorders of the eye, the skin or the lower intestinal tract.

For topical application to the eye, or ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline or, preferably, as a solution in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, the compounds may be formulated into ointments, such as petrolatum.

For topical application to the skin, the compounds can be formulated into suitable ointments containing the compounds suspended or dissolved in, for example, mixtures with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated into suitable lotions or creams containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application to the lower intestinal tract can be effected in rectal suppository formulations (see above) or in suitable enema formulations.

The compounds of the present invention may be administered by a single dose, multiple discrete doses or continuous infusion. Since the compounds are small, easily diffusible and relatively stable, they are well suited to continuous infusion. Pump means, particularly subcutaneous

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pump means, are preferred for continuous infusion.

Compositions and methods of the invention also may utilize controlled release technology. Thus, for example, NAALADase inhibitors may be incorporated into a polymer matrix for controlled release over a period of days. Such controlled release films are well known to the art. Examples of polymers commonly employed for this purpose that may be used in the present invention include nondegradable ethylene-vinyl acetate copolymer and degradable lactic acid-glycolic acid copolymers. Certain hydrogels such as poly(hydroxyethylmethacrylate) or poly(vinylalcohol) also may be useful.

Dosage

Dose levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels being about 0.1 mg to about 1,000 mg. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the rate of excretion; drug combination; the severity of the particular disease being treated; and the form of administration. Typically, in vitro dosage-effect results provide useful guidance on the proper doses for patient administration. Studies in animal models are also helpful, particularly in determining effective doses for treating cancer. The considerations for determining the proper dose levels are well

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known in the art.

In a preferred embodiment, the compounds of the present invention are administered in lyophilized form. In this case, 1 to 100 mg of a compound of the present invention may be
5 lyophilized in individual vials, together with a carrier and a buffer, such as mannitol and sodium phosphate. The compound may be reconstituted in the vials with bacteriostatic water before administration.

As previously mentioned, the compounds of the present
10 invention may be administered in combination with one or more therapeutic agents, including chemotherapeutic agents. TABLE I provides known median dosages for selected chemotherapeutic agents. Specific dose levels for these agents will depend upon considerations such as those identified above for the
15 compounds of the present invention.

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TABLE I

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
	Asparaginase	10,000 units
	Bleomycin Sulfate	15 units
5	Carboplatin	50-450 mg
	Carmustine	100 mg
	Cisplatin	10-50 mg
	Cladribine	10 mg
10	Cyclophosphamide (lyophilized)	100 mg-2 gm
	Cyclophosphamide (non-lyophilized)	100 mg-2 gm
	Cytarabine (lyophilized powder)	100 mg-2 gm
15	Dacarbazine	100 mg-200 mg
	Dactinomycin	0.5 mg
	Daunorubicin	20 mg
	Diethylstilbestrol	250 mg
	Doxorubicin	10-150 mg
20	Etidronate	300 mg
	Etoposide	100 mg
	Floxuridine	500 mg
	Fludarabine Phosphate	50 mg
	Fluorouracil	500 mg-5 gm
25	Goserelin	3.6 mg
	Granisetron Hydrochloride	1 mg
	Idarubicin	5-10 mg
	Ifosfamide	1-3 gm
	Leucovorin Calcium	50-350 mg
30	Leuprolide	3.75-7.5 mg
	Mechlorethamine	10 mg
	Medroxyprogesterone	1 gm
	Melphalan	50 gm
	Methotrexate	20 mg-1 gm

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CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
Mitomycin	5-40 mg
Mitoxantrone	20-30 mg
Ondansetron Hydrochloride	40 mg
Paclitaxel	30 mg
Pamidronate Disodium	30-*90 mg
Pegaspargase	750 units
Plicamycin	2,500 mcgm
Streptozocin	1 gm
Thiotepa	15 mg
Teniposide	50 mg
Vinblastine	10 mg
Vincristine	1-5 mg
Aldesleukin	22 million units
Epoetin Alpha	2,000-10,000 units
Filgrastim	300-480 mcgm
Immune Globulin	500 mg-10 gm
Interferon Alpha-2a	3-36 million units
Interferon Alpha-2b	3-50 million units
Levamisole	50 mg
Octreotide	1,000-5,000 mcgm
Sargramostim	250-500 mcgm

Administration regimen

For the methods of the present invention, any administration regimen regulating the timing and sequence of drug delivery can be used and repeated as necessary to effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

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For patients with prostate cancer that is neither advanced nor metastatic, the compounds of the present invention may be administered (i) prior to surgery or radiation treatment to reduce the risk of metastasis; (ii) during surgery or in conjunction with radiation treatment; and/or (iii) after surgery or radiation therapy to reduce the risk of recurrence and to inhibit the growth of any residual tumorous cells.

For patients with advanced or metastatic prostate cancer, the compounds of the present invention may be administered as a continuous supplement to, or as a replacement for, hormonal ablation in order to slow tumor cell growth in both the untreated primary tumor and the existing metastatic lesions.

The methods of the present invention are particularly useful where shed cells could not be removed by surgical intervention. After post-surgical recovery, the methods of the present invention would be effective in reducing the chances of recurrence of a tumor engendered by such shed cells.

Combination with Other Treatments

(i) Surgery and Radiation Treatment

In general, surgery and radiation treatment are employed as potentially curative therapies for patients with localized prostate cancer who are under 70 years of age and are expected to live at least 10 more years.

Approximately 70% of newly diagnosed prostate cancer patients fall into this category. Approximately 90% of these

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patients (65% of total patients) undergo surgery, while approximately 10% of these patients (7% of total patients) undergo radiation treatment.

Histopathological examination of surgical specimens reveals that approximately 63% of patients undergoing surgery (40% of total patients) have locally extensive tumors or regional (lymph node) metastasis that was undetected at initial diagnosis. These patients are at a significantly greater risk of recurrence. Approximately 40% of these patients will actually develop recurrence within five years after surgery. Results after radiation treatment are even less encouraging. Approximately 80% of patients who have undergone radiation treatment as their primary therapy have disease persistence or develop recurrence or metastasis within five years after treatment.

Currently, most prostate cancer patients undergoing surgery and radiation treatment do not receive any immediate follow-up therapy. Rather, they are monitored frequently for elevated Prostate Specific Antigen ("PSA"), which is the primary indicator of recurrence or metastasis.

Based on the above statistics, there is considerable opportunity to use the present invention in conjunction with surgery and/or radiation treatment.

(ii) Hormonal Therapy

Hormonal ablation is the most effective palliative treatment for the 10% of patients with metastatic prostate cancer. Hormonal ablation by medication and/or orchiectomy is used to block hormones that promote further growth and

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metastasis of prostate cancer. With time, both the primary and metastatic tumors of virtually all of these patients become hormone-independent and resistant to therapy. Approximately 50% of patients with metastatic cancer die within three years after initial diagnosis, and 75% of such patients die within five years after diagnosis. Continuous supplementation with the compounds of the present invention may be used to prevent or reverse this potentially metastasis-permissive state.

10 (iii) Chemotherapy

While chemotherapy has been successful in treating some forms of cancer, it has shown slight therapeutic value in treating prostate cancer where it is generally reserved as a last resort. Accordingly, the opportunity to treat prostate cancer by combining chemotherapy with the methods of the present invention will be rare. When combined, however, such treatments should be more effective than chemotherapy alone in controlling prostate cancer.

15 (iv) Immunotherapy

20 The compounds of the present invention may also be used in combination with monoclonal antibodies to treat prostate cancer. Such combined treatment is particularly effective for patients with pelvic lymph node involvement, of which only 34% survive after 5 years. An example of such monoclonal antibodies is cell membrane-specific anti-prostate antibody.

25 The present invention may also be used with immunotherapies based on polyclonal or monoclonal antibody-derived reagents. Monoclonal antibody-derived reagents are

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preferred. These reagents are well known in the art, and include radiolabelled monoclonal antibodies such as monoclonal antibodies conjugated with strontium-89.

(v) Cryotherapy

5 The methods of the present invention may also be used in conjunction with cryotherapy for treatment of prostate cancer.

Experimental Studies

10 The following experimental studies of compounds of the present invention and of structurally related compounds provide strong evidence that the compounds of the present invention are non-toxic and are effective in inhibiting NAALADase activity, treating glutamate abnormalities and treating prostate diseases.

15

In Vivo Toxicity of NAALADase Inhibitors

20 To examine the toxicological effect of NAALADase inhibition *in vivo*, a group of mice were injected with 2- (phosphonomethyl)pentanedioic acid, a NAALADase inhibitor of high activity, in doses of 1, 5, 10, 30, 100, 300 and 500 mg/kg body weight. The mice were subsequently observed two times per day for 5 consecutive days. The survival rate at each dose level is provided in TABLE II below. The results show that the NAALADase inhibitor is non-toxic to mice, suggesting that the compounds of the present invention would be similarly non-toxic to humans when administered at therapeutically effective amounts.

25

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TABLE II

TOXICOLOGICAL EFFECTS OF NAALADASE INHIBITORS							
Dose (mg/kg)	1	5	10	30	100	300	500
Survival Rate After 5 days (%)	100	100	100	100	100	100	66.7

In Vitro Assay of NAALADase Activity

The following compounds were tested for *in vitro* inhibition of NAALADase activity. The results are provided in Tables III(a), III(b), and III(c) below.

TABLE III(a)

IN VITRO ACTIVITY OF NAALADASE INHIBITORS

Compound	K _i (nM)
2-(phosphonomethyl)pentanedioic acid	0.275 ± 0.08
2-(phosphonomethyl)succinic acid	700.00 ± 67.3
2-[[2-carboxyethyl)hydroxyphosphinyl]-methyl]pentanedioic acid)	1.89 ± 0.19

2-(phosphonomethyl)pentanedioic acid showed a high level of NAALADase inhibiting activity, with a K_i of 0.27 nM (Table III(a)). The activity of this compound is >1000 times more potent than that of previously described inhibitors. Since 2-(phosphonomethyl)pentanedioic acid is similar in structure to the compounds of the present invention, the results suggest that the compounds of the present invention would also be potent NAALADase inhibitors. By comparison, 2-(phosphonomethyl)succinic acid exhibits much lower NAALADase

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inhibiting activity, suggesting that a glutamate analog attached to the phosphonic acid contributes to its NAALADase inhibiting activity. The results also show that 2-[(2-carboxyethyl)-hydroxyphosphinyl]methyl]pentanedioic acid, which has an additional carboxylic acid side chain similar to the aspartate residue found in NAAG, exhibits a lower NAALADase inhibiting activity than 2-(phosphonomethyl)-pentanedioic acid.

10

Table III (b)

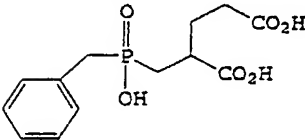
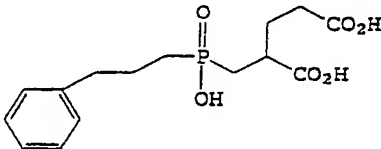
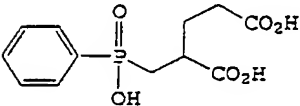
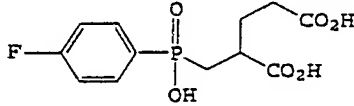
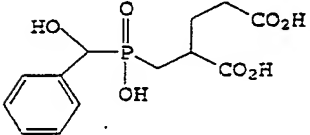
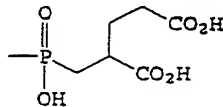
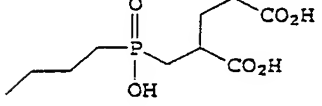
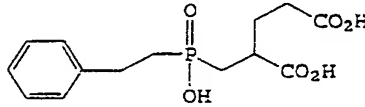
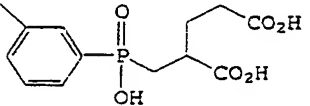
Other compounds demonstrating inhibition of NAALADase activity are set forth below in Table III(b). Results of the nine compounds in Table III(b) shows the remarkable K_i activity of a variety of compounds of the present invention. These compounds show NAALADase inhibitory ability wherein R1 comprises an aliphatic group, a aliphatic which is substituted, an aromatic group, and aromatic which is substituted.

15

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Table III(b)

In vitro Activity of NAALADase Inhibitors

COMPOUND	Ki (nM)	COMPOUND	Ki (nM)
	34		231
	36		532
	54		1100
	148		148
	190		

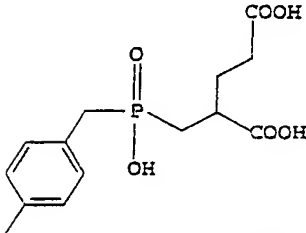
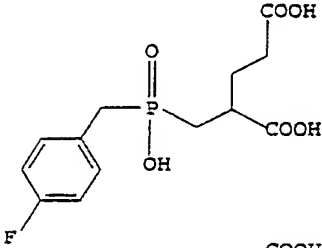
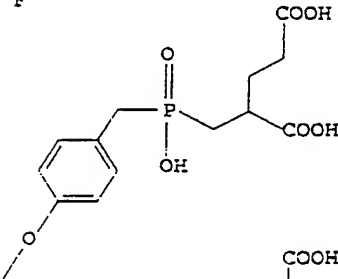
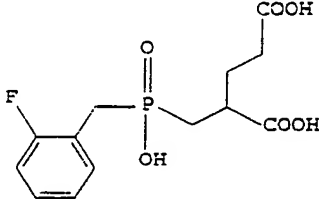
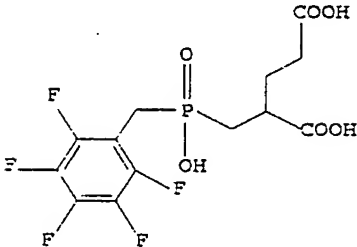
5

Further results, provided in Table III(c), show the remarkable Ki activity of the compounds of the present invention. These compounds show NAALADase inhibition wherein R1 comprises a substituted aliphatic (benzyl) which is further substituted.

10

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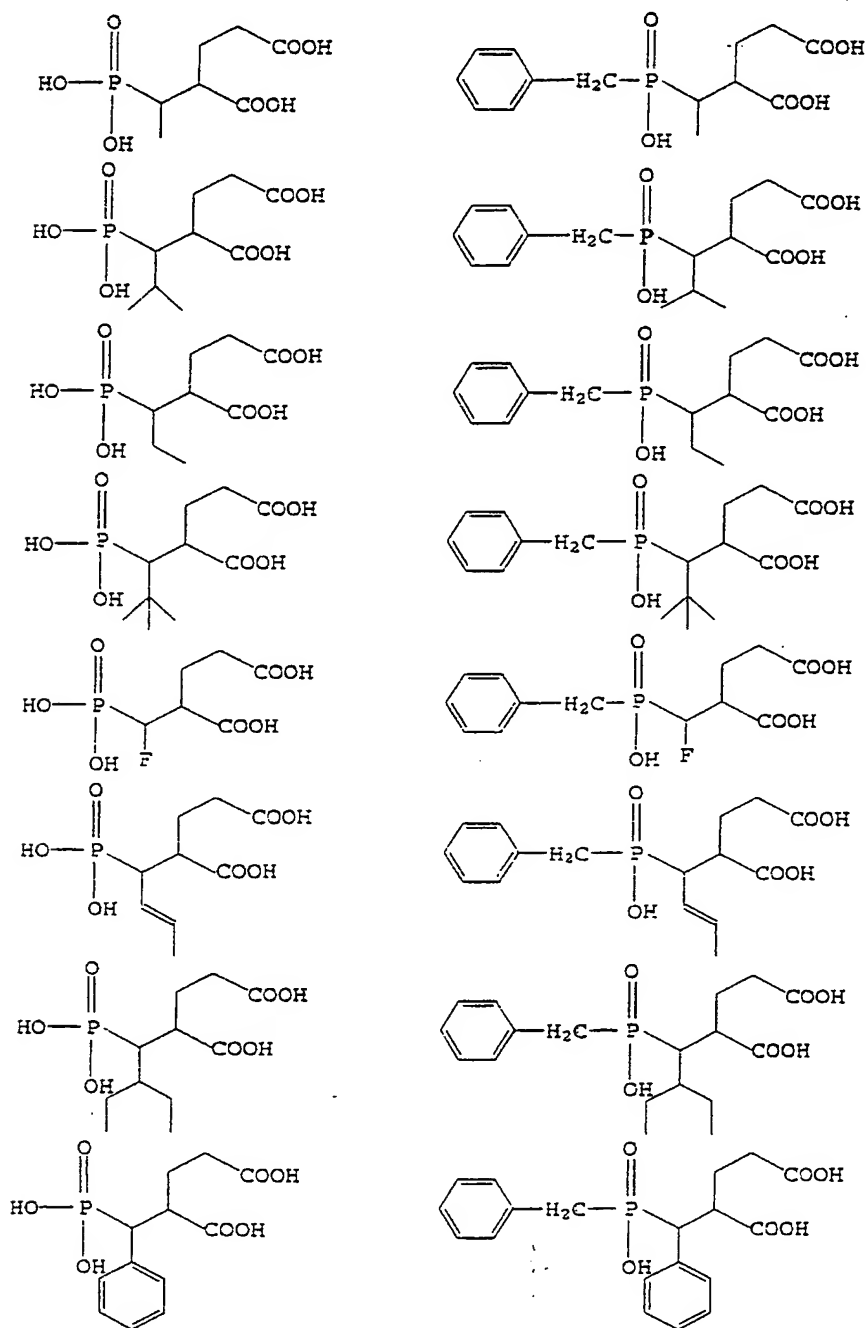
Table III(c) *in vitro* Activity of NAALADase Inhibitors

<u>Compound</u>	<u>K_i Value (nM)</u>
	K _i = 68nM
	K _i = 70nM
	K _i = 90nM
	K _i = 175nM
	K _i = 38nM

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Compounds of the present invention which possess similar NAALADase inhibitory activity may be found below in Table III(d).

Table III(d) Exemplary Compounds of the Present Invention



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Protocol for In Vitro Assay of NAALADase Activity

The amount of [^3H]Glu liberated from [^3H]NAAG in 50 mM Tris-Cl buffer was measured for 15 minutes at 37° C using 30-50 μg of synaptosomal protein. Substrate and product were resolved by anion-exchange liquid chromatography. Duplicate assays were performed so that no more than 20% of the NAAG was digested, representing the linear range of peptidase activity. Quisqualate (100 μM) was included in parallel assay tubes to confirm the specificity of the measurements.

In Vitro Assay of NAALADase Inhibitors
on Cancer

Referring now to FIGS. 1 and 2, the effect of NAALADase inhibitors on cancer cell line were examined. LNCAP cells (a prostate cancer cell line) were treated with quisqualate acid (in concentrations ranging from 10 nM to 1 μM) and 2-(phosphonomethyl)pentanedioic acid (in concentrations ranging from 100 pM to 10 nM). The 3H-thymidine measurement for each concentration of quisqualate acid and 2-(phosphonomethyl)pentanedioic acid is also provided in TABLE IV below. FIGS. 1 and 2 present this data graphically and particularly illustrate the decrease in proliferation and thymidine uptake of cells treated with NAALADase inhibitors.

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TABLE IV

<u>3H-Thymidine Incorporation (dpm/well)</u>			
	<u>Dose</u>	<u>Quisqualic Acid</u>	<u>2-(phosphonomethyl)-pentanedioic acid</u>
5	Control	4813 \pm 572	4299 \pm 887
	10 pM	--	3078 \pm 1006
10	100 pM	--	2062 \pm 595
	1 nM	3668 \pm 866	1001 \pm 52
	10 nM	2137 \pm 764	664 \pm 366
15	100 nM	1543 \pm 312	--
	1 μ M	1295 \pm 181	--

20

The results show that LNCAP cell proliferation (as measured by the incorporation of 3H-thymidine) decreased significantly as the concentration of the NAALADase inhibitors increased, suggesting that the compounds of the present invention would be effective in treating cancer, particularly prostate cancer.

25

Protocol for In Vitro Cancer Assay

Cells in RPMI 1640 medium containing 10% Fetal Calf Serum (FCS) are plated in 24 well plates and allowed to adhere for 24 hours before addition of quisqualic acid (10^{-9} M to 10^{-6} M) or 2-(phosphonomethyl)pentanedioic acid (10^{-11} M to 10^{-8} M) for 7 days. On the 7th day, the cells are pulsed with 3H-thymidine for 4 hours, harvested and measured for radioactivity. Values represent means \pm SEM of 6 separate cell wells for each treatment. All experiments are performed

30

35

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at least twice.

To control for non-specific cytostatic effects of quisqualate acid and 2-(phosphonomethyl)pentanedioic acid, the agents are simultaneously evaluated on a non-NAALADase containing prostate cell line, DU145 (Carter et al., Proc. Natl. Acad. Sci. USA, (93) 749-753, 1996). If the treatments with quisqualate acid and 2-(phosphonomethyl)pentanedioic have no significant effect on cell growth, the NAALADase inhibiting activity of the agents are uniquely responsible for their cytostatic effects on NAALADase containing prostate carcinoma cell lines.

Cell Lines and Tissue Culture

LNCAP cells are obtained from Dr. William Nelson at the Johns Hopkins School of Medicine in Baltimore, MD. DU145 cells are obtained from American Type Culture Collection (Rockville, MD). Cells are grown in RPMI-1640 media supplemented with 10% heat-inactivated fetal calf serum, 2 mM-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin (Paragon) in a humidified incubator at 37°C in a 5% CO₂/95% O₂ atmosphere.

[3H] Thymidine Incorporation Assays

The cells are suspended at 1×10^3 cells/ml in RPMI-1640 media and seeded into 24-well plates at 500 µl per well. After 24 hours, various concentrations of quisqualic acid (Sigma) or the potent NAALADase inhibitor 2-(phosphonomethyl)pentanedioic acid (synthesized according to the methods of Jackson et al., J Med Chem 39(2) 619-622) is added to the wells and the plates are returned to the

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incubator. On days 3, 5 and 7, media and drug are refreshed. On the 8th day following seeding, each well is pulsed with 1 μ Ci 3 H-thymidine (New England Nuclear) for 4 hours. Media is then removed and the wells washed 2 times with phosphate buffered saline (pH=7.4). The contents of each well is subsequently solubilized with 250 μ l of 0.2 N NaOH and transferred to scintillation vials. 5 ml UltimaGold (Packard) scintillation cocktail is added and radioactivity is quantitated using a Beckman LS6001 scintillation counter.

The purity and/or identity of all synthetic compounds is ascertained by thin layer chromatography, High Pressure Liquid Chromatography (HPLC), mass spectrometry, and elemental analysis. Proton Nuclear Magnetic Resonance (NMR) spectra are obtained using a Bruker spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. Analytical thin-layer chromatography (TLC) is conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates is accomplished by using UV light, phosphomolybdic acid-ethanol, and/or iodoplatinate charring. Flash chromatography is conducted on Kieselgel 60, 230-400 mesh (E. Merck, Darmstadt, West Germany). Solvents are either reagent or HPLC grade. Reactions are run at ambient temperature and under a nitrogen atmosphere unless otherwise noted. Solutions are evaporated under reduced pressure on a Buchi rotary evaporator.

In vivo LNCaP Tumor Xenograft Assay and Results

Referring now to FIGS. 3 and 4, LNCaP human prostate cancer

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cells were injected subcutaneously into the right flank of male nude mice. 2-(phosphonomethyl)pentanedioic acid, a NAALADase inhibitor, was administered by daily intratumoral injection (0.25 μ g/day) beginning when the tumors reached a volume of approximately 50-70 mm³. An additional group was included using a silicon polymer containing 2-(phosphonomethyl)pentanedioic acid which released approximately 0.25 μ g/day of drug locally into the tumor. The 2-(phosphonomethyl)pentanedioic acid polymer was changed two times per week. Tumor volumes were monitored for 42 days after the beginning of treatment.

EXPERIMENTAL PROCEDURES

Cell Lines

LNCaP is a human prostate cancer cell line that was established in 1973 from a pleural effusion of a patient who had been treated with 5-FU, doxorubicin, methotrexate, and CTX in the 3 months before the cell line was initiated. This line is androgen receptor positive and has been used in screening anticancer drugs that are targeted as hormone antagonists. LNCaP was grown in RPMI with 1.5 g NaHCO₃/L, 10% fetal bovine serum (FBS), and 2 mM L-glutamine and was kept at 37°C in a humidified 5% CO₂/O₂ incubator. Antibiotics were not added to the medium.

Animal Tumor Model

NCr nude (nu/nu) male mice, age 4-5 weeks, were purchased from Taconic (Germantown, NY). The animals were housed four per cage in sterile filter-topped cages in a ventilated cage rack. Upon arrival, they were quarantined for four working days before

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use. Temperature was maintained at $72 \pm 5^{\circ}\text{F}$ and relative humidity at 35-70%, and a 12-hr light/dark cycle is used. The mice were fed sterile, autoclavable, certified Purina rodent chow *ad libitum*. Drinking water was acidified and autoclaved, and the source water was recirculated, deionized, UV-treated, and 5- μm filtered.

After the animals were released from quarantine, the mice were injected subcutaneously in the right flank with 1×10^7 LNCaP cells in Matrigel™ (0.1-ml injection volume). Tumor dimensions and body weight were measured twice weekly. Vernier calipers were used to measure tumors in three planes, and tumor volume (V) was calculated as follows: $V = \pi(x \times y \times z)/6$, where x, y, and z were the tumor measurements minus skin thickness. At the end of the experiment, the mice were sacrificed by CO₂ inhalation followed by cervical dislocation.

Pharmaceuticals

2-(phosphonomethyl)pentanedioic acid was made up in water at a concentration of 2.5 mg/ml. Polymer containing 2-(phosphonomethyl)pentanedioic acid was made up by grinding 140 mg NaCl to a fine powder then mixing with 5mg 2-(phosphonomethyl)pentanedioic acid and 350 mg silicone gel. The mixture was spread to a thin film and allowed to dry for 24 hours. The material was cut into 1-1.5 mg pieces for subcutaneous implantation.

Treatment Protocol

When the tumor volumes reached a predetermined size (mean

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tumor volume 50-70 mm³), mice were added randomly into treatment groups of six to eight mice each. All treatments were administered daily for at least 4 weeks. 2-(phosphonomethyl)pentanedioic acid was administered intratumorally daily in a volume of 0.05 ml containing 0.025 µg 2-(phosphonomethyl)pentanedioic acid per injection.

Polymer containing 2-(phosphonomethyl)pentanedioic acid (10 µg drug/mg polymer) was implanted subcutaneously. Mice were anaesthetized with metaflane, and a small (<2mm) incision was made near the tumor site. Following implantation, the incision was closed with a wound clip. Polymer was replaced twice weekly.

The tumor were measured twice weekly for at least 8 weeks after the first treatment. The mean tumor volume for each group was calculated for each time point. Comparisons between groups at specific times were made using an unpaired, two-tailed t-test, and the results were analyzed using analysis of variance (ANOVA).

Systemic toxicity was assessed from reductions in body weight after treatment. The mice were sacrificed at the end of the follow-up period, or earlier if their tumor volumes reached 1600 mm³ or the tumors ulcerated.

Statistical Analysis

Statistical analysis as described above was performed using JMP (SAS Institute Inc., Cary, NC)

In vivo Rat Dunning R3327 Model

Referring now to FIGS. 5 and 6, Dunning R3327-G prostate cancer cells were injected subcutaneously into both flanks of

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syngeneic male rats. In the first study, the anti-tumor growth activity of 2-(phosphonomethyl)pentanedioic acid was tested following daily subcutaneous injections of the drug (1,3 10 and 30 mg/kg). 2-(phosphonomethyl)pentanedioic acid injections and
5 tumor measurements were continued for 12 weeks. In the second study, the anti-tumor growth activity of 2-[[phenylmethyl)hydroxyphosphinyl)methyl]pentanedioic acid was tested following daily intra-tumoral injections of the drug (0.1,1,10,100 μ g) after the tumor reached an initial volume of
10 80-290 mm³. Tumor volumes were subsequently monitored for 42 days after the beginning of drug treatment.

EXPERIMENTAL PROCEDURES

Cell Lines

15 R3327-G is a cell line derived from an androgen-sensitive papillary adenocarcinoma derived from a spontaneously forming tumor in the rat prostate. R3327-G cells were grown in RPMI, 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 10⁻⁸ M dexamethasone. Cultures were kept at 37°C in a humidified 5%
20 CO₂/O₂ incubator. Antibiotics were not added to the medium.

Animal Tumor Model

Copenhagen male rats, age 8-10 weeks, were purchased from Harlan Sprague Dawley (Indianapolis, IN). The animals were
25 housed two per cage. Upon arrival, they were quarantined for four working days before use. Temperature was maintained at 72 \pm 5°F and relative humidity at 35-70%, and a 12-hr light/dark cycle was used. The rats were fed certified Purina rodent chow

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and water *ad libitum*.

After the animals were released from quarantine, the rats were injected subcutaneously in both flanks with 1×10^7 R3327-G cells (0.1-ml injection volume). Tumor dimensions and body weight were measured twice weekly. Vernier calipers were used to measure tumors in three planes, and tumor volume (V) was calculated as follows: $V = \pi(x \times y \times z)/6$, where x, y, and z were the tumor measurements minus skin thickness. Tumors began to appear 4-5 weeks after tumor cell injection. At the end of the experiment, the rats were sacrificed by CO₂ inhalation.

Pharmaceuticals

2-(phosphonomethyl)pentanedioic acid was made up in physiological saline fresh each day prior to injection. A stock solution of 2-[[phenylmethyl)hydroxyphosphinyl]methyl]pentanedioic acid was made up in water at a concentration 2.5 mg/ml; ten-fold serial dilutions were made fresh weekly for injections.

Treatment Protocol

In the 2-(phosphonomethyl)pentanedioic acid study, the rats were given daily subcutaneous injections of drug beginning the 14 days following tumor cell implantation and continued for 12 weeks. In the 2-[[phenylmethyl)hydroxyphosphinyl]methyl]pentanedioic acid study, the drug was not administered until the tumor volumes reached a predetermined size (mean tumor volume 90-290 mm³). At this time, the rats were divided into treatment groups of five rats each. All treatments of 2-

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[[phenylmethyl)hydroxyphosphinyl)methyl] pentanedioic acid were subsequently administered intra-tumorally daily for 6 weeks.

The tumors were measured twice weekly. The mean tumor volume for each group was calculated for each time point.

5 Comparisons between groups at specific times were made using an unpaired, two-tailed t-test, and the results were analyzed using analyzed of variance (ANOVA). For the 2-

[[phenylmethyl)hydroxyphosphinyl)methyl] pentanedioic acid study, individual tumor volumes (V) were expressed as a fraction of the

10 tumor volume on Day 0, the first day of treatment (V0). For each

group, the mean of the ratio V/V0 was plotted as a function of time after treatment.

Statistical Analysis

15 Statistical analysis as described above was performed using JMP (SAS Institute, Inc. Cary, NC).

EXAMPLES

20 The following examples are illustrative of preferred embodiments of methods of use and preparation of compounds of the invention and are not to be construed as limiting the invention thereto. Unless otherwise indicated, all percentages are based upon 100% of the final formulations.

EXAMPLE 1

25 Preparation of 2-[(methvlhydroxyphosphinyl)methyl]pentanedioic acid

Scheme IV R=CH3, R1=CH2Ph

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Methyl-O-benzylphosphinic acid

Dichloromethylphosphite (10.0 g, 77 mmol) in 80 mL of dry diethyl ether was cooled to -20°C under an atmosphere of nitrogen. A solution of benzyl alcohol (23 g, 213 mmol) and triethylamine (10.2 g, 100 mmol) in 40 mL of diethyl ether was added dropwise over 1 hour while maintaining an internal temperature range of 0°C to 10°C. Once addition was complete the mixture was warmed to room temperature and stirred overnight. The mixture was filtered and the solid cake washed with 200 mL of diethyl ether. The organics were combined and evaporated under reduced pressure to give 25 g of a clear and colorless liquid. The liquid was purified by flash chromatography and eluted with a 1:1 hexane/ethyl acetate to ethyl acetate gradient. The desired fractions were collected and evaporated to give methyl O-benzylphosphinic acid (1, R=CH₃, R₁=CH₂Ph, 6.5 g, 50%) as a clear and colorless oil. R_f 0.1 (1:1, Hexane/EtOAc).
¹H NMR(d₆-DMSO): 7.4 ppm (m, 5H), 7.1 ppm (d, 1H), 5.0 ppm (dd, 2H), 1.5 ppm (d, 3H)

20 2,4-Di(benzoyloxycarbonyl)butyl(methyl)-O-benzylphosphinic acid

Methyl-O-benzylphosphinic acid (3.53 g, 20.7 mmol) in 200 mL of dichloromethane was cooled to -5°C under an atmosphere of nitrogen. Triethylamine (3.2 g, 32 mmol) was added via syringe followed by trimethylsilyl chloride (2.9 g, 27 mmol). The reaction mixture was stirred and warmed to room temperature over 1 hour. Dibenzyl 2-methylenepentanedioate (2, 6.0 g, 18.5 mmol) in 10 mL of dichloromethane was added. The mixture was then stirred at room temperature overnight. The reaction mixture was

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cooled to 0°C and trimethylaluminum (9 mL, 18 mmol, 2.0 M in dichloromethane) was added. The flask was warmed and stirred for 72 hours. The clear light yellow solution was cooled to 5°C and quenched by the slow addition of 5% hydrochloric acid. The quenched reaction mixture was warmed to room temperature and the organic layer removed. The organic layer was washed with 5% hydrochloric acid and with water. The organics were dried (MgSO₄) and evaporated under reduced pressure to give 8 g of a clear light yellow oil. The oil was purified on silica gel and eluted with a gradient of 1:1 hexanes/ethyl acetate to 100% ethyl acetate. The desired fractions were collected and evaporated to give 2,4-di(benzyloxycarbonyl)buty(methyl)-O-benzylphosphinic acid (3, R=CH₃, R₁=CH₂Ph 0.8 g, 8%) as a clear and colorless oil. R_f 0.5 (ethyl acetate).

¹H NMR (CDCl₃): 7.4 ppm (m, 15H), 5.1 ppm (m, 6H), 3.0 ppm (m, 1H), 2.4 ppm (m, 3H), 2.1 ppm (m, 3H), 1.5 ppm (dd, 3H)

Elemental Analysis Calculated C₂₈H₃₁O₆P.0.5H₂O: C 68.01, H 6.32

Found: C 66.85, H 6.35

20 2-[(Methylhydroxyphosphinyl)methyl]pentanedioic acid

2,4-di(benzyloxycarbonyl)buty(methyl)-O-benzylphosphinic acid (0.8 g, 1.6 mmol) in 20 mL of water containing 100 mg of 10% Pd/C was hydrogenated at 40 psi for 4 hours. The mixture was filtered over a pad of Celite and evaporated at high vacuum to give 2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid (4, R=CH₃, 0.28 g, 78% as a clear and colorless viscous oil.

¹H NMR (D₂O): 2.5 ppm (m, 1H), 2.2 ppm (t, 2H), 2.0 ppm (m, 1H), 1.7 ppm (m, 3H), 1.3 ppm (d, 3H)

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Elemental Analysis Calculated C₇H₁₁O₆P.0.2 H₂O: C36.92 H 5.93

Found: C37.06 H 6.31

EXAMPLE 25 Preparation of 2-[(butylhydroxyphosphinyl)methyl]pentanedioic acid

Scheme IV R=n-butyl, R1=H

Butylphosphinic Acid

10 Diethyl chlorophosphite (25g, 0.16mol) in 60 mL of dry ether was cooled to 0°C under an atmosphere of nitrogen.

Butylmagnesium chloride (80 mL, 0.16 mol, 2.0 M solution in ether) was added dropwise over a period of 2 hours while maintaining the internal temperature at 0°C. Once addition

15 was complete the thick white slurry was heated to 30°C for 1 hour. The suspension was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure. The clear light yellow liquid was then brought up in 15 mL of water and stirred at room temperature. Concentrated hydrochloric acid

20 (0.5 mL) was then added and an exothermic reaction was observed. The mixture was stirred an additional 15 minutes and extracted with two 75 mL portions of ethyl acetate. The organics were combined, dried (MgSO₄) and evaporated to give a clear and colorless liquid. The liquid was treated with NaOH

25 (40 mL, 20 M) and stirred for 1 hour. The mixture was then washed with diethyl ether and acidified to pH 1.0. The desired material was extracted from the acidified extract with two 100 mL portions of ethyl acetate. The organics were

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combined, dried (MgSO_4) and evaporated under reduced pressure to give butylphosphinic acid (1, R=n-butyl, R1=H, 10g, 51%) as a clear and colorless liquid.

^1H NMR (d_6 -DMSO): 6.9 ppm(d, 1H), 1.6 ppm(m, 2H), 1.4 ppm(m, 4H), 0.9 ppm(t, 3H)

Butyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid

Butylphosphinic acid (2.0g, 16mmol) in 80 mL of dry dichloromethane was cooled to 0°C under an atmosphere of nitrogen. Triethylamine (6.7 g, 66 mmol) was added followed by trimethylsilyl chloride (58 mL, 58 mmol, 1.0 M in dichloromethane). The mixture was stirred at 0°C for 10 minutes and dibenzyl 2-methylenepentanedioate (2) (6.4 g, 20 mmol) in 20 mL of dichloromethane was added. The cold bath was removed and the reaction warmed to room temperature and stirred overnight. The mixture was then cooled to 0°C and quenched by the slow addition of 5% hydrochloric acid. The dichloromethane layer was then removed and washed with 5% hydrochloric acid and with brine. The organic layer was dried (MgSO_4) and evaporated to give a clear light golden liquid. The liquid was purified by flash chromatography and eluted with 3:1 hexane/ethyl acetate containing 5% acetic acid. The desired fractions were combined and evaporated to give butyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (3, R=n-butyl, R1=H) (2.9 g, 40%) as a clear and colorless oil. Rf 0.12 (3:1, Hex./EtOAc 5% AcOH).

^1H NMR (d_6 -DMSO): 7.3 ppm (m, 10), 5.0 ppm (s, 4H), 2.7 ppm (m, 1H) 2.3 ppm (y, 2H), 1.8 ppm (m, 2H), 1.3 ppm (m, 4H), 0.8 ppm

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(t, 3H)

2-[(Butylhydroxyphosphinyl)methyl]pentanedioic acid

Butyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (2.9 g,
5 6.5 mmol) in 30 mL of water containing 0.32 g 10% Pd/C was
hydrogenated on a Parr hydrogenator at 40 psi for 4.5 hours.
The mixture was filtered through a pad of Celite and
evaporated under high vacuum to give 2-

[(butylhydroxyphosphinyl)methyl]pentanedioic acid (4, R=n-
10 butyl) (0.75 g, 43%) as a clear and colorless viscous oil.
¹H NMR (D₂O): 2.4 ppm (m, 1H), 2.1 ppm (t, 2H), 1.9 ppm (m,
1H), 1.6 ppm (m, 3H), 1.4 ppm (m, 2H), 1.1 ppm (m, 4H), 0.6
ppm (t, 3H)

Elemental Analysis Calculated C₁₀H₁₉O₅P. 0.5 H₂O: C 43.64, H
15 7.32: Found C 43.25, H 7.12

EXAMPLE 3Preparation of 2-[(benzylhydroxyphosphinyl)methyl]pentanedioic
acid

20 Scheme IV R=CH₂Ph, R₁=H

Benzylphosphinic acid

Diethylchlorophosphite (25 g, 0.16 mol) in 100 mL of dry
diethyl ether was cooled to 0°C under an atmosphere of
25 nitrogen. Benzylmagnesium chloride (80 mL, 0.16 mol, 2.0 M
solution in Et₂O) was added dropwise over two hours while
maintaining a temperature below 10°C. A thick white slurry
formed and stirring was continued at room temperature for 1

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hour. The mixture was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure to give a clear and colorless liquid. The liquid was stirred as 15 mL of water was added followed by 0.5ml concentrated hydrochloric acid. An exothermic reaction was observed and stirring was continued for an additional 30 minutes followed by extraction with ethyl acetate. The organics were combined, washed with brine, dried (MgSO_4) and evaporated. The clear light golden liquid was added to sodium hydroxide (50 mL, 2.0 M NaOH), stirred for one hour and washed with diethyl ether. The aqueous layer was acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate. The organics were combined, dried (MgSO_4) and evaporated to give benzylphosphinic acid (1, $\text{R}=\text{CH}_2\text{Ph}$, R_1+H) (8 g, 32%) as a clear light golden oil.

^1H NMR (d_6 -DMSO): 7.3 ppm (m, 5H), 6.9 ppm (d, 1H), 3.1 ppm (d, 2H)

Benzyl[2,4-di(benzylloxycarbonyl)butyl]phosphinic acid

Benzylphosphinic acid (2.3 g, 15 mmol) in 150 mL of dry dichloromethane was cooled to 0°C under a nitrogen atmosphere. Triethylamine (6.5 g, 65mmol) was added followed by trimethylsilyl chloride (5.8 g, 54 mmol) while the reaction temperature was maintained at 0°C . After 30 minutes dibenzyl 2-methylenepentanedioate (2) in 20 mL of dichloromethane was added over 5 minutes. The reaction mixture was left to warm to room temperature and stirred overnight. The clear solution was cooled to 0°C and quenched with 5% hydrochloric acid and

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with brine, dried (MgSO_4) and evaporated to give a clear yellow liquid. Purification by flash chromatography and elution with 1:1 hexane/ethyl acetate containing 10% acetic acid yielded 2.0 g (28%) of benzyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (3, $\text{R}=\text{CH}_2\text{Ph}$, $\text{R}_1=\text{H}$) as a clear light yellow oil. R_f 0.37 (1:1 Hex./EtOAc, 10%AcOH).

^1H NMR (d_6 -DMSO): 7.2 ppm(m, 15H), 5.0 ppm(s, 4H), 3.0 (d, 2H), 2.8 ppm(m, 1H), 2.3 ppm(t, 2H), 1.9 ppm(m, 2H), 1.7 ppm(t, 1H)

2-[(benzylhydroxyphosphinyl)methyl]pentanedioic acid

Benzyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (0.5 g, 1.0 mmol) in 20 mL of water containing 120 mg of 10% Pd/C was hydrogenated on a Parr hydrogenator at 40 psi for 6 hours.

Filtration through a Celite pad followed by evaporation on high vacuum gave 0.17 g (57%) of 2-[(benzylhydroxyphosphinyl)methyl]pentanedioic acid (4, $\text{R}=\text{CH}_2\text{Ph}$) as a white solid.

^1H NMR (D_2O): 7.1 ppm(m, 5H), 2.9 ppm(d, 2H), 2.4 ppm(m, 1H), 2.1 ppm(t, 2H), 1.8 ppm(m, 1H), 1.6 ppm(m, 3H)

Elemental Analysis, Calculated $\text{C}_{13}\text{H}_{17}\text{O}_6\text{P}$: C52.00H5.71: Found: C51.48H5.70

EXAMPLE 4

Preparation of 2-

[(phenylethylhydroxyphosphinyl)methyl]pentanedioic acid

Scheme IV $\text{R}=\text{CH}_2\text{CH}_2\text{Ph}$, $\text{R}_1=\text{H}$

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Phenethylphosphinic acid

Diethylchlorophosphite (15.6 g, 0.1 mol) in 100 mL of dry diethyl ether was cooled to 5°C under an atmosphere of nitrogen. Phenethylmagnesium chloride (100 mL, 0.1 mol, 1.0 M in THF) was added dropwise over 2 hours while maintaining a temperature between 0-10°C. A thick white slurry formed and stirred at room temperature overnight. The mixture was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure to give a clear and colorless liquid. The liquid was stirred as 15 mL of water was added followed by 0.5 mL of concentrated hydrochloric acid. An exothermic reaction was observed and stirring continued for 15 minutes followed by extraction with ethyl acetate. The organics were combined, washed with brine, dried (MgSO₄) and evaporated. The clear liquid was brought up in sodium hydroxide (40 mL, 2.0 M NaOH), stirred for 1 hour and washed once with diethyl ether. The aqueous layer was acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate. The organics were combined, dried (MgSO₄) and evaporated to give phenethylphosphinic acid (1, R=CH₂CH₂Ph, R₁=H) (9.8 g, 58%) as a clear light yellow oil.

¹H NMR (d₆-DMSO): 7.2 ppm (m, 5H), 6.9 ppm (d, 1H), 2.8 ppm (m, 2H), 1.9 ppm (m, 2H)

2,4-Di(benzoyloxycarbonyl)butyl(phenethyl)phosphinic acid

Phenethylphosphinic acid (1.0 g, 5.9 mmol) in 50 mL of dry dichloromethane was cooled to -5°C under a nitrogen

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atmosphere. Triethylamine (2.3g, 23 mmol) was added followed by trimethylsilyl chloride 2.2 g, 21 mmol) while the reaction temperature was maintained at 0°C. After 10 minutes dibenzyl 2-methylenepentanedioate (2) in 10 mL of dichloromethane was added over 10 minutes. The reaction mixture was left to warm to room temperature and stirred overnight. The clear solution was cooled to 0°C and quenched with 5% hydrochloric acid followed by removal of the organic layer. The organic layer was washed with brine, dried (MgSO₄) and evaporated to give a clear light golden liquid. Purification by flash chromatography and elution with 1:1 Hexane/EtOAc containing 5% AcOH resulted in 1.2g (41%) of 2,4-di(benzyloxycarbonyl)butyl(phenethyl)phosphinic acid(3,R=CH₂CH₂Ph,R₁=H) as a clear and colorless oil.

¹H NMR (d₆-DMSO): 7.2 ppm(m,15H), 5.0 ppm (s,4H), 3.3 ppm (m,1H), 2.8 ppm(m,4H), 2.3 ppm(m,2), 1.8 ppm(m,4H)

2,4-[(Phenethylhydroxyphosphinyl)methyl]pentanedioic acid

2,4-Di(benzyloxycarbonyl)butyl(phenethyl)phosphinic acid(1.1 g,2.2 mmol) in 20 mL of water containing 120 mg of 10% Pd/C was hydrogenated on a Parr hydrogenator at 40 psi overnight. Filtration through a Celite pad followed by evaporation on high vacuum gave 0.8 g (114%) of 2-

[(phenethylhydroxyphosphinyl)methyl]pentanedioic acid(4,R=CH₂CH₂Ph) as a white solid.

¹H NMR(D₂O): 7.2 ppm (m,5H), 2.7 ppm (m,2H), 2.5 ppm (m,1H), 2.3 ppm (t,2H), 1.9 ppm (m,6H), 1.5 ppm (t,1H)

Elemental Analysis:

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Calculated $C_{14}H_{19}O_5P \cdot 0.75H_2O \cdot 0.5 AcOH$: C 50.35 H 6.34

Found: C 50.26 H 5.78

EXAMPLE 5

5 Preparation of 2-[(3-
 phenylpropylhydroxyphosphinyl)methyl]pentanedioic acid
Scheme IV R=CH₂CH₂CH₂Ph, R₁=H

3-Phenylpropylphosphinic acid

10 Magnesium turnings (2.44 g, 0.10 mol) in 20 mL of dry diethyl
 ether under an atmosphere of nitrogen was added several iodine
 crystals. Phenylpropyl bromide (20.0 g, 0.10 mol) in 80 mL of
 diethyl ether was placed in a dropping funnel. Approximately
 10 mL of the bromide solution was added to the magnesium
15 turnings and stirring was initiated. After several minutes
 the iodine was consumed and additional phenylpropyl bromide
 was added while maintaining a temperature of 35°C. Once
 additional was complete (1.5 hours) the mixture was sealed and
 stored at 5°C.

20 Diethylchlorophosphite (15.7 g, 0.1 mol) in 50 mL of dry
 diethyl ether was cooled to 5°C under an atmosphere of
 nitrogen. Phenylpropylmagnesium bromide (100 mL, 0.1 mol, 1.0
 M solution of in Et₂O) was added dropwise over 2 hours while
 maintaining a temperature between 0-10°C. A thick white
25 slurry formed and was stirred an additional 30 minutes. The
 mixture was filtered under a nitrogen atmosphere and the
 filtrate evaporated under reduced pressure to give a clear and
 colorless liquid. To the liquid was added 20 mL of water

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followed by 0.5ml of concentrated hydrochloric acid. An exothermic reaction was observed and stirring continued for 20 minutes followed by extraction with ethyl acetate. The organics were combined, washed with brine, dried (MgSO_4) and evaporated. To the clear liquid was added sodium hydroxide (40 mL, 2.0 M NaOH), the resulting solution stirred for 1 hour and then washed with diethyl ether. The aqueous layer was acidified to pH 1.0 with concentrated hydrochloric acid and extracted twice with ethyl acetate. The organics were combined, dried (MgSO_4) and evaporated to give 3-phenylpropylphosphinic acid (1, $\text{R}=\text{CH}_2\text{CH}_2\text{CH}_2\text{Ph}$, $\text{R}_1=\text{H}$) (9.8 g, 53%) as a clear and colorless oil.

^1H NMR (d_6 -DMSO): 7.2 ppm (m, 5H), 6.9 ppm (d, 1H), 2.6 ppm (t, 2H), 1.7 ppm (m, 2H), 1.6 ppm (m, 2H)

2,4-Di(benzoyloxycarbonyl)butyl(3-phenylpropyl)phosphinic acid
3-phenylpropylphosphinic acid (1.0 g, 5.4 mmol) in 50 mL of dry dichloromethane was cooled to -5°C under a nitrogen atmosphere. Triethylamine (2.2 g, 22 mmol) was added followed by trimethylsilyl chloride (2.1 g, 19 mmol) while the reaction temperature was maintained at 0°C . After 10 minutes dibenzyl 2-methylenepentanedioate (2) in 10 mL of dichloromethane was added over 10 minutes. The reaction mixture was warmed to room temperature and stirred overnight. The clear solution was cooled to 0°C and quenched with 5% hydrochloric acid followed by removal of the organic layer. The organic layer was washed with brine, dried (MgSO_4) and evaporated to give a clear yellow liquid. Purification by flash chromatography and

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elution with 4:1 hexane/ethyl acetate containing 5% acetic acid resulted in 1.5g (56%) of 2,4-di(benzyloxycarbonyl)butyl(3-phenylpropyl)phosphinic acid(3,R=CH₂CH₂CH₂Ph, R₁=H) as a clear light yellow oil. R_f 0.58 (1:1 Hex./EtOAc, 5%AcOH);

¹H NMR (d₆-DMSO): 7.2 ppm (m, 15H), 5.0 ppm (s, 4H), 2.7 ppm (m, 1H), 2.5 ppm (m, 5H), 2.2 ppm (m, 2H), 1.8 ppm (m, 3H), 1.6 ppm (m, 2H)

Elemental Analysis:

Calculated C₂₉H₃₃O₆P. 1.3H₂O: C 65.48 H 6.75

Found: C 65.24 H 6.39

2-[(3-Phenylpropylhydroxyphosphinyl)methyl]pentanedioic acid

2,4-Di(benzyloxycarbonyl)butyl(3-phenylpropyl)phosphinic acid(15) (1.4 g, 2.8 mmol) in 20 mL of water containing 150 mg of 10% Pd/C was hydrogenated on a Parr hydrogenator at 40 psi overnight. Filtration through a Celite pad followed by evaporation on high vacuum gave 0.8 g (89%) of 2-[(3-phenylpropylhydroxyphosphinyl)methyl] pentanedioic acid(4,R=CH₂CH₂CH₂Ph) as a light yellow viscous oil).

¹H NMR (D₂O): 7.4 ppm (m, 5H), 2.7 ppm (m, 3H), 2.4 ppm (t, 3H), 1.8 ppm (m, 7H);

Elemental Analysis:

Calculated C₁₅H₂₁O₆P 0.75 H₂O, 0.75 AcOH: C 51.23 H 6.64

Found: C 50.85 H 6.02

EXAMPLE 6

Preparation of 2-[(4-methylbenzyl)hydroxyphosphinyl]

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methyllpentanedioic acid

Scheme V, Compound 5

Hexamethyldisilazane (21.1 mL, 100 mmol) was added to vigorously stirred ammonium phosphinate (8.30 g, 100 mmol), and the resulting suspension was stirred at 105 C for 2 h. A solution of 4-methylbenzyl bromide (5.00 g, 27.0 mmol) was then dropwise added to the suspension at 0°C. The mixture was stirred at rt for 19 h. The reaction mixture was then diluted with dichloromethane (50 mL) and washed with 1 N HCl (50 mL). The organic layer was separated, dried over Na₂SO₄, and concentrated to give 4.72 g of a white solid. This was dissolved in dichloromethane (50 mL) and benzyl alcohol (3.24 g, 30 mmol) was added to the solution. 1,3-Dicyclohexylcarbodiimide (DCC) (6.19 g, 30 mmol) was then added to the solution at 0°C, and the suspension was stirred at rt for 14 h. The solvent was removed under reduced pressure and the residue was suspended in EtOAc. The resulting suspension was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (hexanes: EtOAc, 4:1 to 1:1) to give 2.40 g of 4-methylbenzyl-O-benzylphosphinic acid (2, R=4-methylbenzyl) as a white solid (34% yield): R_f 0.42 (EtOAc); ¹H NMR (DMSO-d₆) delta 2.30 (s, 3 H), 3.29 (d, J = 16.6 Hz, 2 H), 5.2 (m, 2 H), 7.0 (d, J = 543 Hz, 1 H), 7.1-7.2 (m, 4 H), 7.3-7.4 (m, 5 H).

To a solution of 4-methylbenzyl-O-benzylphosphinic acid (2, R=4-methylbenzyl) (2.16 g, 8.3 mmol) in THF (15 mL) was added sodium hydride (0.10g, 60 % dispersion in oil) followed by

-85-

dibenzyl 2-methylenepentanedioate at 0 C, and the mixture was stirred at rt for 4 h. The reaction mixture was then diluted with EtOAc (50 mL) and poured into 1N HCl (50 mL). The organic layer was separated, dried over Na₂SO₄, and concentrated. This material was purified by silica gel chromatography (hexanes: EtOAc, 4:1 to 1:1) to give 3.41 g of 2,4-di(benzyloxycarbonyl)butyl(4-methylbenzyl)-o-benzylphosphinic acid (4, R = 4-methylbenzyl) as colorless oil (70% yield): R_f 0.61 (EtOAc); ¹H NMR (CDCl₃) delta 1.6-1.8 (m, 1 H), 1.9-2.0 (m, 2 H), 2.1-2.4 (m, 6 H), 2.7-2.9 (m, 1 H), 3.05 (dd, J = 9.0, 16.8 Hz, 2 H), 4.8-5.1 (m, 6 H), 7.0-7.1 (m, 4 H), 7.2-7.4 (m, 15 H).

To a solution of 2,4-di(benzyloxycarbonyl)butyl(4-methylbenzyl)-o-benzylphosphinic acid (0.70 g, 1.2 mmol) in ethanol (30 mL) was added Pd/C (5%, 0.10 g) and the suspension was shaken under hydrogen (50 psi) for 18 h. The suspension was then filtered through a pad of Celite and concentrated under reduced pressure. The resulting residue was dissolved in distilled water (5 mL), passed through a column of AG 50W-X8 resin (H⁺ form), and lyophilized to give 0.21 g of 2-[[[(4-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic acid (5, R = 4-methylbenzyl) as a white solid (55% yield): R_f 0.62 (i-PrOH:H₂O, 7:3); ¹H NMR (D₂O) delta 1.7-1.9 (m, 3 H), 2.0-2.2 (m, 1 H), 2.33 (dt, J = 1.7 Hz, 7.4 Hz, 2 H), 2.55-2.70 (m, 1 H), 3.12 (d, J = 16.5 Hz, 2 H), 7.0-7.1 (m, 2 H), 7.2-7.3 (m, 2 H). Anal. Calcd for C₁₃H₁₇O₅P·0.30H₂O: C, 52.60; H, 6.18. Found: C, 52.60; H, 6.28.

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EXAMPLE 7Preparation of 2-[[[4-Fluorobenzyl]hydroxyphosphinyl]methylpentanedioic acid (R = 4-fluorobenzyl):

Scheme V, prepared as described in the above example where R =
5 methylbenzyl:

R_f 0.64 (*i*-PrOH:H₂O, 7:3); ¹H NMR (D₂O) delta 1.7-1.9 (m, 3 H),
2.0-2.2 (m, 1 H), 2.3-2.4 (m, 2 H), 2.55-2.70 (m, 1 H), 3.12
(d, *J* = 16.5 Hz, 2 H), 7.0-7.1 (m, 2 H), 7.2-7.3 (m, 2 H).

Anal. Calcd for C₁₃H₁₆FO₅P*0.25H₂O: C, 48.38; H, 5.15. Found: C,
10 48.38; H, 5.15.

EXAMPLE 8Preparation of 2-[[[4-Methoxybenzyl]hydroxyphosphinyl]methylpentanedioic acid (R = 4-methoxybenzyl):

15 Scheme V, prepared as described in the above example where R =
methylbenzyl:

R_f 0.56 (*i*-PrOH:H₂O, 7:3); ¹H NMR (D₂O) delta 1.8-1.9 (m, 3 H),
2.0-2.2 (m, 1 H), 2.3-2.4 (m, 2 H), 2.55-2.70 (m, 1 H), 3.16
(d, *J* = 16.7 Hz, 2 H), 3.81 (s, 3 H), 6.98 (d, *J* = 8.7 Hz, 2

20 H), 7.25 (d, *J* = 8.7 Hz, 2 H). Anal. Calcd for
C₁₄H₁₉O₇P*0.30H₂O: C, 50.09; H, 5.89. Found: C, 49.98; H, 5.80.

EXAMPLE 9Preparation of 2-[[[2-Fluorobenzyl]hydroxyphosphinyl]

25 methylpentanedioic acid (R = 2-fluorobenzyl):

Scheme V, prepared as described in the above example where R
= methylbenzyl:

R_f 0.67 (*i*-PrOH:H₂O, 7:3); ¹H NMR (D₂O) delta 1.8-1.9 (m, 3 H),

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2.0-2.2 (m, 1 H), 2.3-2.4 (m, 2 H), 2.55-2.70 (m, 1 H), 3.28 (d, $J = 16.6$ Hz, 2 H), 7.1-7.5 (m, 4 H). Anal. Calcd for $C_{13}H_{16}FO_6P \cdot 0.10H_2O$: C, 48.79; H, 5.10. Found: C, 48.84; H, 5.14.

5

EXAMPLE 10

Preparation of 2-[(Pentafluorobenzyl)hydroxyphosphinyl]methylpentanedioic acid (R = pentafluorobenzyl):

Scheme V, prepared as described in the above example where R = methylbenzyl:

10 R_f 0.69 (i-PrOH:H₂O, 7:3); ¹H NMR (D₂O) δ 1.8-2.0 (m, 3 H), 2.1-2.3 (m, 1 H), 2.3-2.5 (m, 2 H), 2.7-2.9 (m, 1 H), 3.29 (d, $J = 15.4$ Hz, 2 H), Anal. Calcd for $C_{13}H_{12}F_5O_6P \cdot 0.45H_2O$: C, 39.20; H, 3.26. Found: C, 39.17; H, 3.28.

15

EXAMPLE 11

Preparation of 2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid

Scheme VI, Compound 9

20 2,4-Di(benzyloxycarbonyl)butylphosphinic acid (6)
Dry phosphinic acid (100 g, 1.52 mol) was dissolved in 100 ml of chloroform and treated with triethylamine (155 g, 1.52 mol). The mixture was evaporated and transferred to a three liter flask, containing 750 mL of chloroform. The solution was
25 stirred by means of a mechanical stirrer and the flask cooled to 0°C. The clear solution was treated with triethylamine (277 g, 2.72 mol) followed by trimethylsilyl chloride (281 g, 2.58 mol). Once addition of trimethylsilyl chloride was

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complete dibenzyl 2-methylenepentanedioate (2) in 150 mL of chloroform was added dropwise over 20 minutes. The low temperature bath was removed and the mixture warmed to room temperature. After 6 hours the thick slurry was filtered and the filtrate cooled to 0°C. The filtrate was then quenched with 5% hydrochloric acid and the organic layer removed. The aqueous layer was extracted with chloroform, the organics combined, dried (MgSO₄) and evaporated under reduced pressure to give 55 g of 2,4-di(benzyloxycarbonyl)butylphosphinic acid (6) as a light yellow liquid. The liquid was purified by flash chromatography and eluted using 3:1 hexanes/ethyl acetate containing 5% trifluoroacetic acid to give 40 g (7%) of the desired product. Rf 0.28 (3:1 Hex./EtOAc 5% TFA); ¹H NMR (CDCl₃): 7.3 ppm (m, 10H), 7.2 ppm (d, 1H), 5.12 ppm (s, 2H), 2.9 ppm (m, 1H), 2.4 ppm (t, 2H), 2.2 ppm (m, 1H), 2.0 ppm (m, 3H)

2,4-Di(benzyloxycarbonyl)butylbenzylphosphinic acid (7)

To a solution of 2,4-di-(benzyloxycarbonyl)butyl phosphinic acid (6) (19.3 g, 49.4 mmol) in tetrahydrofuran was added benzyl alcohol (5.3 g, 49.3 mmol) and dimethylamino in tetrahydrofuran was added benzyl alcohol (5.3 g, 49.3 mmol) and dimethylamino pyridine (0.5 g). Dicyclohexylcarbodiimide (DCC, 12g, 58 mmol) was added and a white precipitate formed. After 30 minutes the white suspension was filtered and the filtrate evaporated under reduced pressure. The clear and colorless oil was purified by flash chromatography and eluted with 1:1 Hex./EtOAc to give 2,4-

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di(benzyloxycarbonyl)butylbenzylphosphinic acid (7) (11.5 g, 47%) as a clear and colorless oil. Rf. 0.16 (1:1 Hex./EtOAc); ¹H NMR (CDCl₃): 7.3 ppm (m, 15H), 7.2 ppm (d, 1H), 5.0 ppm (m, 6H), 2.9 ppm (m, 1H), 2.2 ppm (m, 3H), 1.9 ppm (m, 3H)

5

2,4-Di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]benzylphosphinic acid (8)

2,4-Di(benzyloxycarbonyl)butylbenzylphosphinic acid (7) in 5 mL of dry THF was added dropwise to a stirring cooled (0°C) mixture of sodium hydride (0.09 g, 2.3 mmol) in 15 mL of THF. After 15 minutes benzaldehyde (0.23 g, 2.2 mmol) was added via syringe while maintaining a temperature of 0°C. After 30 minutes the mixture was quenched with water and extracted with two portions of dichloromethane. The organics were combined and evaporated to give a clear colorless oil. The oil was chromatographed on silica and eluted with a 1:1 Hex./EtOAc solvent system. The desired fractions were collected and evaporated to give 0.4 g (33%) of 2,4-di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]benzylphosphinic acid (6) as a clear and colorless oil. Rf 0.18 (1:1 Hex./EtOAc);

20

¹H NMR (CDCl₃): 7.3 ppm (m, 20H), 5.2 ppm (m, 1H), 4.9 ppm (m, 6H), 2.8 ppm (dm, 1H), 2.2 ppm (m, 3H), 1.9 ppm (m, 3H)

25

2-([hydroxy(phenyl)methyl]hydroxyphosphinylmethyl)pentanedioic acid(9)

2,4-Di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]benzylphosphinic acid(6) (0.37 g, 0.6 mmol) in 25 mL of

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water containing 0.10g of 10% Pd/C was hydrogenated at 40 psi for 6 hours. The mixture was filtered through a pad of Celite and lyophilized to give 2-

([hydroxy(phenyl)methyl]hydroxyphosphinylmethyl)pentanedioic acid (9) (0.14 g, 70%) as a white solid.

^1H NMR (D₂O): 7.4 ppm (m, 5H), 5.0 ppm (d, 1H), 2.7 ppm (m, 1H), 2.4 ppm (m, 2H), 2.2 ppm (m, 1H), 1.9 ppm (m, 3H)

Element Analysis:

Calculated C₁₃H₁₇O₇P. 0.6H₂O: C 47.74 H 5.61

Found: C 47.73 H 5.68

EXAMPLE 12

Preparation of Dibenzyl 2-Methylenepentanedioate.

Scheme III.

Benzyl acrylate (500 g, 3 mol) was heated to 100°C under an atmosphere of nitrogen. The heating was stopped and HMPT (10 g, 61 mmol) was added dropwise while maintaining an internal temperature of 135-145°C. Once addition was complete the mixture was cooled to room temperature and a slurry of silica with 5:1 Hex/EtOAc was added. The slurry was then transferred to a column containing a plug of dry silica. The column was then washed with 1:1 Hex/EtOAc and the solvent was collected and evaporated. The clear yellow liquid was distilled under high vacuum (200 μHg) to give an initial fraction of 8 g distilling at 45°C and then the desired product at 180-185°C (212 g, 42 %) as a clear and colorless liquid.

^1H -NMR (CDCl₃)

7.3 ppm (s, 10H); 6.2 ppm (s, 1H); 5.5 ppm (s, 1H); 5.2 ppm

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(s, 2H); 5.1 ppm (s, 2H); 2.6 ppm (m, 4H).

EXAMPLE 13

Preparation of Dibenzyl 2-[[Bis(benzylloxyl)phosphoryl]methyl]-pentanedioate.

5

Scheme III

Dibenzyl phosphite (9.5g, 36mmol) in 350ml of dichloromethane was cooled to 0°C. To this stirring solution was added trimethyl aluminum (18.2ml, 2.0M solution in hexane, 36.4mmol). After 30 minutes 1 (6.0g, 37 mmol) in 90 ml of dichloromethane was added dropwise over 10 minutes. The clear and colorless solution was then warmed to room temperature and left to stir overnight. The mixture was then quenched by the slow addition of 5% HCl. After stirring an additional 1.5 hours the lower organic layer was removed and the aqueous layer extracted once with 100ml of dichloromethane. The organics were combined, dried (MgSO₄), and evaporated to give a clear light golden liquid. The liquid was chromatographed on silica gel (4cm*30cm) and eluted with a gradient (4:1-1:1) solvent system (Hexane/EtOAc). The fractions containing the desired product were combined and evaporated to yield 2 (7.1g, 42%) as a clear and colorless liquid. The liquid was then distilled on a Kugleror apparatus at 0.5mm Hg and 195-200°C. The distillate was discarded and the remaining light golden oil was chromatographed on silica gel (1:1, Hex./EtOAc) to give 2.9g of 2 as a clear and colorless oil. TLC R_f 0.5 (1:1, Hex./EtOAc).

¹H-NMR (CDCl₃)

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7.1-7.4 (m, 20H); 5.05 (s, 2H); 4.8-5.03 (m, 6H); 2.8 (1H);
2.22-2.40 (m, 3H); 1.80-2.02 (m, 3H).

EXAMPLE 145 Preparation of 2-(Phosphonomethyl)pentanedioic Acid.

Scheme III

The benzyl pentanedioate (2.9g, 4.9mmol) was added to a mixture of 20ml of methanol containing 0.29g (6mol%) of 10% Pd/C. This mixture was hydrogenated on a Parr hydrogenator at 40 psi for 24 hours, filtered and evaporated to give 3 (1.0g, 90%) as a clear slightly golden viscous oil.

¹H-NMR (D₂O)

2.6-2.78 (m, 1H); 2.25-2.40 (m, 2H); 1.75-2.15 (m, 4H).

15

EXAMPLE 15

A patient is diagnosed with adenocarcinoma of the prostate. The patient may then be administered a NAALADase inhibitor, such as set forth in examples 1 through 3, by direct injection into the tumor. After this initial treatment, the patient may be optionally administered the same or different NAALADase inhibitor by intermittent or continuous administration by subdural pump. It would be expected that no further occurrences of the adenocarcinoma would develop.

20

EXAMPLE 16

A patient is diagnosed with adenocarcinoma of the prostate. The patient may then be administered a NAALADase inhibitor, such as set forth in examples 1 through 3, by direct injection into the tumor. After this initial

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treatment, the patient may be optionally administered the same or different NAALADase inhibitor by intermittent or continuous administration by implantation of a biocompatible, polymeric matrix delivery system. It would be expected that no further occurrences of the adenocarcinoma would develop.

EXAMPLE 17

A patient is diagnosed with benign prostatic hyperplasia. The patient may then be administered a NAALADase inhibitor, such as set forth in examples 1 through 3, by direct injection into the tumor. After this initial treatment, the patient may be optionally administered the same or different NAALADase inhibitor by intermittent or continuous administration by injection, subdural pump, or polymeric matrix implant. It would be expected that the benign prostatic hyperplastic cells do not develop into carcinoma.

EXAMPLE 18

A patient is diagnosed with adenocarcinoma of the prostate. The adenocarcinoma appears not to have metastasized. The adenocarcinoma would be removed by surgery. After post-operative recovery, the patient would be locally administered NAALADase inhibitor by intermittent or continuous administration by injection, subdural pump or by polymeric matrix implant. It would be expected that no further occurrences of the carcinoma would develop.

EXAMPLE 19

A patient is diagnosed with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears to have metastasized, but surgery still is indicated as an effective

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treatment modality. Tumor tissue would be removed by surgery. The patient would be locally administered a NAALADase inhibitor such as described herein from the time, approximately, of the initial diagnosis and would continue after surgery. After post-operative recovery, the patient would be maintained at this level of NAALADase inhibitor by a regimen of periodic local administration. The patient would be monitored carefully for intolerable adverse side-effects of NAALADase inhibitor administration. It would be expected that no further tumors develop. If some of the original, small tumorous masses are detected after surgery, they would be expected to not grow in size.

EXAMPLE 20

A patient is diagnosed with ACTH-producing tumors. The patient may then be administered a NAALADase inhibitor, such as set forth in examples 1 through 3, by direct injection into the tumor. After this initial treatment, the patient may be optionally administered the same or different NAALADase inhibitor by direct injection, subdural pump, or implantation of a biocompatible, polymeric matrix delivery system. It would be expected that tumor growth or tumor cell growth would be prevented or inhibited and that no further occurrences of the ACTH-producing tumor would develop.

EXAMPLE 21

A treatment such as that described in Example 9 wherein the patient is diagnosed with acute lymphocytic leukemia.

EXAMPLE 22

A treatment such as that described in Example 9 wherein

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the patient is diagnosed with acute non-lymphocytic leukemia.

EXAMPLE 23

5 A treatment such as that described in Example 9 wherein
the patient is diagnosed with metastatic or non-metastatic
cancer of the adrenal cortex.

EXAMPLE 24

10 A treatment such as that described in Example 9 wherein
the patient is diagnosed with metastatic or non-metastatic
bladder cancer.

EXAMPLE 25

A treatment such as that described in Example 9 wherein
the patient is diagnosed with metastatic or non-metastatic
brain cancer.

15 EXAMPLE 26

A treatment such as that described in Example 9 wherein
the patient is diagnosed with metastatic or non-metastatic
breast cancer.

EXAMPLE 27

20 A treatment such as that described in Example 9 wherein
the patient is diagnosed with metastatic or non-metastatic
cervical cancer.

EXAMPLE 28

25 A treatment such as that described in Example 9 wherein
the patient is diagnosed with metastatic or non-metastatic
chronic lymphocytic leukemia.

EXAMPLE 29

A treatment such as that described in Example 9 wherein

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the patient is diagnosed with metastatic or non-metastatic chronic myelocytic leukemia.

EXAMPLE 30

5 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic colorectal cancer.

EXAMPLE 31

10 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic cutaneous T-cell lymphoma.

EXAMPLE 32

15 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic endometrial cancer.

EXAMPLE 33

20 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic esophageal cancer.

EXAMPLE 34

25 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic Ewing's sarcoma.

EXAMPLE 35

30 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic gallbladder cancer.

EXAMPLE 36

A treatment such as that described in Example 9 wherein

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the patient is diagnosed with metastatic or non-metastatic hairy cell leukemia.

EXAMPLE 37

5 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic head and neck cancer.

EXAMPLE 38

10 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic Hodgkin's lymphoma.

EXAMPLE 39

15 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic Kaposi's sarcoma.

EXAMPLE 40

15 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic kidney cancer.

EXAMPLE 41

20 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic liver cancer.

EXAMPLE 42

25 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic lung cancer (small cell and/or non-small cell).

EXAMPLE 43

A treatment such as that described in Example 9 wherein

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the patient is diagnosed with metastatic or non-metastatic malignant peritoneal effusion.

EXAMPLE 44

5 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic malignant pleural effusion.

EXAMPLE 45

10 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic melanoma.

EXAMPLE 46

15 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic mesothelioma.

EXAMPLE 47

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic multiple myeloma.

20 EXAMPLE 48

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic neuroblastoma.

EXAMPLE 49

25 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic non-Hodgkin's lymphoma.

EXAMPLE 50

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A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic osteosarcoma.

EXAMPLE 51

5 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic ovarian cancer (and/or germ cell ovarian cancer).

EXAMPLE 52

10 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic pancreatic cancer.

EXAMPLE 53

15 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic penis cancer.

EXAMPLE 54

20 A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic retinoblastoma.

EXAMPLE 55

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic skin cancer.

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EXAMPLE 56

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic soft-tissue sarcoma.

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EXAMPLE 57

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic squamous cell carcinoma.

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EXAMPLE 58

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic stomach cancer.

EXAMPLE 59

10

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic testicular cancer.

EXAMPLE 60

15

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic thyroid cancer.

EXAMPLE 61

20

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic trophoblastic neoplasm.

EXAMPLE 62

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic uterine cancer.

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EXAMPLE 63

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic vaginal cancer.

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EXAMPLE 64

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic cancer of the vulva.

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EXAMPLE 65

A treatment such as that described in Example 9 wherein the patient is diagnosed with metastatic or non-metastatic Wilm's tumor.

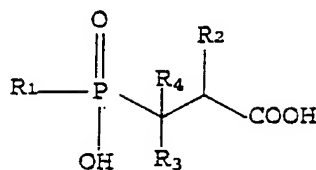
10

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modification are intended to be included within the scope of the following claims.

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What is claimed is:

1. A compound of the formula:



I

5 wherein

R_1 is hydrogen, C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl group, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, or Ar_1 ;

10 R_2 is C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl group, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, or Ar_1 , wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl or aryl group may be optionally substituted with carboxylic acid;

15 R_3 and R_4 are independently hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, dialkyl, halogen, or Ar_1 provided that both R_3 and R_4 are not hydrogen,

20 wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl or aryl groups of R_1 and R_2 may be optionally substituted with C_3 - C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5 - C_7 cycloalkenyl, C_1 - C_4 alkyl, C_2 - C_4 alkenyl, halo, hydroxy, carboxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_4 alkoxy, C_2 - C_4 alkenyloxy, phenoxy, 25 benzyloxy, or Ar_1 , and where Ar_1 is selected from the group

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consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, 2-thienyl, 3-thienyl, 4-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to five substituents which are independently selected from the group consisting of hydrogen, halo, hydroxy, carboxy, 5 nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, and benzyloxy; or a pharmaceutically acceptable salt, hydrate, or a mixture thereof.

10 2. A pharmaceutical composition comprising an effective amount of a compound of claim 1 and a pharmaceutically acceptable carrier.

15 3. A method of treating cancer in an animal comprising administering to said animal an effective amount of a compound of claim 1.

4. The method of claim 3 wherein the compound is administered in combination with an additional therapeutic agent selected from the group consisting of: therapeutic hormones, chemotherapeutic hormones, anti-angiogenesis agents, radiolabelled compounds, and mixtures thereof. 20

5. The method of claim 3 wherein the cancer is selected from the group consisting of: brain cancer, cancer of the adrenal cortex, kidney cancer, testicular cancer, and prostatic adenocarcinoma.

25 6. A compound selected from the group consisting of:
2-[1-[methylhydroxyphosphinyl]ethyl]pentanedioic acid;
2-[1-[ethylhydroxyphosphinyl]propyl]pentanedioic acid;
2-[1-[propylhydroxyphosphinyl]butyl]pentanedioic acid;

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- 2-[1-[butylhydroxyphosphinyl]but-2-enyl]pentanedioic acid;
2-[1-[cyclohexylhydroxyphosphinyl]pentyl]pentanedioic acid;
2-[1-[(cyclohexyl)methylhydroxyphosphinyl]hexyl]pentanedioic acid;
5 2-[1-[phenylhydroxyphosphinyl]heptyl]pentanedioic acid;
2-[1-[phenylhydroxyphosphinyl]-1-fluoromethyl]pentanedioic acid;
2-[2-[benzylhydroxyphosphinyl]propyl]pentanedioic acid;
2-[1-[benzylhydroxyphosphinyl]-1-phenylmethyl]pentanedioic acid;
10 2-[1-[phenylethylhydroxyphosphinyl]ethyl]pentanedioic acid;
2-[1-[phenylpropylhydroxyphosphinyl]propyl]pentanedioic acid;
2-[1-[phenylbutylhydroxyphosphinyl]butyl]pentanedioic acid;
2-[1-[(4-methylbenzyl)hydroxyphosphinyl]but-3-enyl]
15 pentanedioic acid;
2-[1-[(4-fluorobenzyl)hydroxyphosphinyl]pentyl]pentanedioic acid;
2-[1-[(2-fluorobenzyl)hydroxyphosphinyl]hexyl]pentanedioic acid;
20 2-[1-[(pentafluorobenzyl)hydroxyphosphinyl]heptyl]pentanedioic acid;
2-[2-[(methoxybenzyl)hydroxyphosphinyl]butyl]pentanedioic acid;
2-[1-[(2, 3, 4-trimethoxyphenyl)hydroxyphosphinyl]hexyl]
25 pentanedioic acid;
2-[1-[(1-naphthyl)hydroxyphosphinyl]heptyl]pentanedioic acid;
2-[1-[(1-naphthyl)hydroxyphosphinyl]-1-fluoromethyl]
pentanedioic acid;

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2-[2-[(2-naphthyl)hydroxyphosphinyl]butyl]pentanedioic acid;

2-[1-[(1-naphthyl)hydroxyphosphinyl]-1-phenylmethyl]

pentanedioic acid;

2-[1-[(1-naphthyl)methylhydroxyphosphinyl]ethyl]pentanedioic

5 acid;

2-[1-[(2-naphthyl)methylhydroxyphosphinyl]ethyl]pentanedioic

acid;

2-[1-[(1-naphthyl)ethylhydroxyphosphinyl]butyl]pentanedioic

acid;

10 2-[1-[(2-naphthyl)ethylhydroxyphosphinyl]but-3-enyl]

pentanedioic acid;

2-[1-[(1-naphthyl)propylhydroxyphosphinyl]pentyl]pentanedioic

acid;

2-[1-[(2-naphthyl)propylhydroxyphosphinyl]hexyl]pentanedioic

15 acid;

2-[1-[(1-naphthyl)butylhydroxyphosphinyl]heptyl]pentanedioic

acid;

2-[2-[(2-naphthyl)butylhydroxyphosphinyl]pentyl]pentanedioic

acid;

20 2-[1-[(phenylprop-2-enyl)hydroxyphosphinyl]ethyl]pentanedioic

acid;

2-[1-[(2-fluorobenzyl)hydroxyphosphinyl]hexyl]pentanedioic

acid;

2-[1-[(hydroxy)phenylmethyl]hydroxyphosphinyl]propyl]

25 pentanedioic acid;

2-[1-[(3-methylbenzyl)hydroxyphosphinyl]butyl]pentanedioic

acid;

2-[1-[(4-fluorophenyl)hydroxyphosphinyl]ethyl]pentanedioic

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- acid;
- 2-(1-phosphonobut-2-enyl)pentanedioic acid;
- 2-[1-[(3-trifluoromethylbenzyl)hydroxyphosphinyl]pentyl]
pentanedioic acid;
- 5 3-(methylhydroxyphosphinyl)-3-ethyl-2-phenylpropanoic acid;
- 3-(ethylhydroxyphosphinyl)-3-propyl-2-phenylpropanoic acid;
- 3-(propylhydroxyphosphinyl)-3-prop-2-enyl-2-phenylpropanoic
acid;
- 3-(butylhydroxyphosphinyl)-3-t-butyl-2-phenylpropanoic acid;
- 10 3-(cyclohexylhydroxyphosphinyl)-3-pentyl-2-phenylpropanoic
acid;
- 3-((cyclohexyl)methylhydroxyphosphinyl)-3-hexyl-2-phenyl
propanoic acid;
- 3-((cyclohexyl)methylhydroxyphosphinyl)-3-fluoro-2-phenyl
15 propanoic acid;
- 3-(phenylhydroxyphosphinyl)-3-methyl-3-butyl-2-phenylpropanoic
acid;
- 3-(phenylhydroxyphosphinyl)-2,3-diphenylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-methyl-2-phenylpropanoic acid;
- 20 3-(phenylethylhydroxyphosphinyl)-3-ethyl-2-phenylpropanoic
acid;
- 3-(phenylpropylhydroxyphosphinyl)-3-propyl-2-phenyl propanoic
acid;
- 3-(phenylbutylhydroxyphosphinyl)-3-prop-1-enyl-2-phenyl
25 propanoic acid;
- 3-((2,3,4-trimethoxyphenyl)-3-hydroxyphosphinyl)-3-t-butyl-
2-phenylpropanoic acid;
- 3-((1-naphthyl)hydroxyphosphinyl)-3-pentyl-2-phenylpropanoic

-107-

acid;

3-((2-naphthyl)hydroxyphosphinyl)-3-hexyl-2-phenyl propanoic
acid;

5 3-((1-naphthyl)methylhydroxyphosphinyl)-3-methyl-3-pentyl-2-
phenyl propanoic acid;

3-((2-naphthyl)methylhydroxyphosphinyl)-3-methyl-2-phenyl
propanoic acid;

3-((1-naphthyl)ethylhydroxyphosphinyl)-3-ethyl-2-phenyl
propanoic acid;

10 3-((2-naphthyl)ethylhydroxyphosphinyl)-3-propyl-2-phenyl
propanoic acid;

3-((1-naphthyl)propylhydroxyphosphinyl)-3-prop-2-enyl-2-phenyl
propanoic acid;

15 3-((2-naphthyl)propylhydroxyphosphinyl)-3-butyl-2-phenyl
propanoic acid;

3-((1-naphthyl)butylhydroxyphosphinyl)-3-pentyl-2-phenyl
propanoic acid;

3-((2-naphthyl)butylhydroxyphosphinyl)-3-hexyl-2-phenyl
propanoic acid;

20 3-(phenylprop-2-enylhydroxyphosphinyl)-3-methyl-3-hexyl-2-
phenylpropanoic acid;

2-[1-(benzylhydroxyphosphinyl)propyl]pentanedioic acid;

2-[1-(methylhydroxyphosphinyl)propyl]hexanedioic acid;

2-[1-(benzylhydroxyphosphinyl)butyl]hexanedioic acid;

25 2-[1-(methylhydroxyphosphinyl)but-2-enyl]heptanedioic acid;

2-[1-(benzylhydroxyphosphinyl)pentyl]heptanedioic acid;

2-[1-(methylhydroxyphosphinyl)hexyl]octanedioic acid;

2-[1-(benzylhydroxyphosphinyl)heptyl]octanedioic acid;

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- 2-[1-(benzylhydroxyphosphinyl)-1-fluoromethyl]octanedioic acid;
- 2-[3-(methylhydroxyphosphinyl)pentyl]nonanedioic acid;
- 2-[1-(methylhydroxyphosphinyl)-1-phenylmethyl]nonanedioic acid;
- 5 2-[1-(benzylhydroxyphosphinyl)ethyl]nonanedioic acid;
- 2-[1-(methylhydroxyphosphinyl)propyl]decanedioic acid;
- 2-[1-(benzylhydroxyphosphinyl)butyl]decanedioic acid;
- 3-(benzylhydroxyphosphinyl)-3-prop-2-enyl-2-methylpropanoic acid;
- 10 3-(benzylhydroxyphosphinyl)-3-butyl-2-ethylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-pentyl-2-propylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-hexyl-2-butylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-fluoro-2-butylpropanoic acid;
- 15 3-(benzylhydroxyphosphinyl)-3-ethyl-3-propyl-2-cyclohexyl propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-phenyl-2-cyclohexylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-methyl-2-(cyclohexyl)methyl propanoic acid;
- 20 3-(benzylhydroxyphosphinyl)-3-ethyl-2-phenylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-propyl-2-benzylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-prop-1-enyl-2-phenylethyl propanoic acid;
- 25 3-(benzylhydroxyphosphinyl)-3-butyl-2-phenylpropylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-pentyl-2-phenylbutylpropanoic acid;

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- 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2, 3, 4-trimethoxyphenyl)propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-ethyl-3-prop-1-enyl-2-(1-naphthyl)propanoic acid;
- 5 3-(benzylhydroxyphosphinyl)-3-methyl-2-(2-naphthyl)propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-ethyl-2-(1-naphthyl)methylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-propyl-2-(2-naphthyl)methylpropanoic acid;
- 10 3-(benzylhydroxyphosphinyl)-3-prop-2-enyl-2-(1-naphthyl)ethylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(2-naphthyl)ethylpropanoic acid;
- 15 3-(benzylhydroxyphosphinyl)-3-pentyl-2-(1-naphthyl)propylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2-naphthyl)propylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-fluoro-2-(2-naphthyl)propylpropanoic acid;
- 20 3-(benzylhydroxyphosphinyl)-3-ethyl-3-butyl-2-(1-naphthyl)butylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-phenyl-2-(1-naphthyl)butylpropanoic acid;
- 25 3-(benzylhydroxyphosphinyl)-3-methyl-2-(2-naphthyl)butylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-ethyl-2-phenylprop-2-enylpropanoic acid;

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- 2-[1-[(2-pyridyl)methylhydroxyphosphinyl]butyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)methylhydroxyphosphinyl]but-2-enyl]pentanedioic acid;
- 5 2-[1-[(4-pyridyl)methylhydroxyphosphinyl]pentyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)ethylhydroxyphosphinyl]hexyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)propylhydroxyphosphinyl]heptyl]pentanedioic acid;
- 10 2-[1-[(3-pyridyl)propylhydroxyphosphinyl]-1-fluoromethyl]pentanedioic acid;
- 2-[3-[(tetrahydrofuranyl)methylhydroxyphosphinyl]octyl]pentanedioic acid;
- 15 2-[1-[(tetrahydrofuranyl)methylhydroxyphosphinyl]-1-phenylmethyl]pentanedioic acid;
- 2-[1-[(tetrahydrofuranyl)ethylhydroxyphosphinyl]ethyl]pentanedioic acid;
- 2-[1-[(tetrahydrofuranyl)propylhydroxyphosphinyl]propyl]pentanedioic acid;
- 20 2-[1-[(2-indolyl)methylhydroxyphosphinyl]butyl]pentanedioic acid;
- 2-[1-[(3-indolyl)methylhydroxyphosphinyl]but-3-enyl]pentanedioic acid;
- 25 2-[1-[(4-indolyl)methylhydroxyphosphinyl]pentyl]pentanedioic acid;
- 2-[1-[(3-indolyl)ethylhydroxyphosphinyl]hexyl]pentanedioic acid;

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- 2-[1-[(3-indolyl)propylhydroxyphosphinyl]heptyl]pentanedioic acid;
- 2-[3-[(2-thienyl)methylhydroxyphosphinyl]nonyl]pentanedioic acid;
- 5 2-[1-[(3-thienyl)methylhydroxyphosphinyl]ethyl]pentanedioic acid;
- 2-[1-[(4-thienyl)methylhydroxyphosphinyl]propyl]pentanedioic acid;
- 2-[1-[(3-thienyl)ethylhydroxyphosphinyl]butyl]pentanedioic acid;
- 10 2-[1-[(3-thienyl)propylhydroxyphosphinyl]but-2-enyl]pentanedioic acid;
- 3-[(2-pyridyl)methylhydroxyphosphinyl]-3-t-butyl-2-phenyl propanoic acid;
- 15 3-[(3-pyridyl)methylhydroxyphosphinyl]-3-pentyl-2-phenyl propanoic acid;
- 3-[(4-pyridyl)methylhydroxyphosphinyl]-3-hexyl-2-phenyl propanoic acid;
- 3-[(4-pyridyl)methylhydroxyphosphinyl]-3-fluoro-2-phenyl propanoic acid;
- 20 3-[(3-pyridyl)ethylhydroxyphosphinyl]-3-dipropyl-2-phenyl propanoic acid;
- 3-[(3-pyridyl)ethylhydroxyphosphinyl]-2,3-diphenylpropanoic acid;
- 25 3-[(3-pyridyl)propylhydroxyphosphinyl]-3-methyl-2-phenyl propanoic acid;
- 3-[(tetrahydrofuranyl)methylhydroxyphosphinyl]-3-ethyl-2-phenylpropanoic acid;

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3-[(tetrahydrofuranyl)ethylhydroxyphosphinyl]-3-propyl-2-phenylpropanoic acid;

3-[(tetrahydrofuranyl)propylhydroxyphosphinyl]-3-prop-2-enyl-2-phenylpropanoic acid;

5 3-[(2-indolyl)methylhydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic acid;

3-[(3-indolyl)methylhydroxyphosphinyl]-3-pentyl-2-phenylpropanoic acid;

3-[(4-indolyl)methylhydroxyphosphinyl]-3-hexyl-2-phenylpropanoic acid;

3-[(3-indolyl)ethylhydroxyphosphinyl]-3-propyl-3-t-butyl-2-phenylpropanoic acid;

3-[(3-indolyl)propylhydroxyphosphinyl]-3-methyl-2-phenylpropanoic acid;

15 3-[(2-thienyl)methylhydroxyphosphinyl]-3-ethyl-2-phenylpropanoic acid;

3-[(3-thienyl)methylhydroxyphosphinyl]-3-propyl-2-phenylpropanoic acid;

3-[(4-thienyl)methylhydroxyphosphinyl]-3-prop-1-enyl-2-phenylpropanoic acid;

3-[(3-thienyl)ethylhydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic acid;

3-[(3-thienyl)propylhydroxyphosphinyl]-3-pentyl-2-phenylpropanoic acid;

25 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2-pyridyl)methylpropanoic acid;

3-(benzylhydroxyphosphinyl)-3-fluoro-2-(2-pyridyl)methylpropanoic acid;

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- 3-(benzylhydroxyphosphinyl)-3-propyl-3-pentyl-2-(3-pyridyl)
methylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-phenyl-2-(3-pyridyl)methyl
propanoic acid;
- 5 3-(benzylhydroxyphosphinyl)-3-methyl-2-(4-pyridyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-ethyl-2-(3-pyridyl)ethyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-propyl-2-(3-pyridyl)propyl
10 propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-prop-2-enyl-2-
(tetrahydrofuranyl)methylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(tetrahydrofuranyl)
ethylpropanoic acid;
- 15 3-(benzylhydroxyphosphinyl)-3-pentyl-2-(tetrahydrofuranyl)
propylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(2-indolyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-propyl-3-hexyl-2-(3-indolyl)
20 methylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-methyl-2-(4-indolyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-ethyl-2-(3-indolyl)ethyl
propanoic acid;
- 25 3-(benzylhydroxyphosphinyl)-3-propyl-2-(3-indolyl)propyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-prop-1-enyl-2-(2-thienyl)
methylpropanoic acid;

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- 3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(3-thienyl)methyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-pentyl-2-(4-thienyl)methyl
propanoic acid;
- 5 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(3-thienyl)ethyl
propanoic acid;
- 3-(benzylhydroxyphosphinyl)-3-t-butyl-3-pentyl-2-(3-thienyl)
propylpropanoic acid;
- 2-[2-(benzylhydroxyphosphinyl)propyl]pentanedioic acid;
- 10 2-[1-(phenylhydroxyphosphinyl)heptyl]pentanedioic acid;
- 2-[1-[(hydroxy)phenylmethyl]hydroxyphosphinyl]but-2-enyl]
pentanedioic acid;
- 2-[1-(butylhydroxyphosphinyl)pentyl]pentanedioic acid;
- 2-[1-[(3-methylbenzyl)hydroxyphosphinyl]hexyl]pentanedioic
15 acid;
- 2-[1-(3-phenylpropylhydroxyphosphinyl)heptyl]pentanedioic
acid;
- 2-[1-(3-phenylpropylhydroxyphosphinyl)-1-fluoromethyl]
pentanedioic acid;
- 20 2-[2-[(4-fluorophenyl)hydroxyphosphinyl]pent-3-enyl]
pentanedioic acid;
- 2-[1-[(4-fluorophenyl)hydroxyphosphinyl]-1-phenylmethyl]
pentanedioic acid;
- 2-[1-(methylhydroxyphosphinyl)ethyl]pentanedioic acid;
- 25 2-[1-(phenylethylhydroxyphosphinyl)propyl]pentanedioic acid;
- 2-[1-[(4-methylbenzyl)hydroxyphosphinyl]butyl]pentanedioic
acid;
- 2-[1-[(4-fluorobenzyl)hydroxyphosphinyl]but-3-enyl]

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- pentanedioic acid;
- 2-[1-[(4-methoxybenzyl)hydroxyphosphinyl]pentyl]pentanedioic acid;
- 2-(2-phosphonopent-4-enyl)pentanedioic acid;
- 5 2-[1-[(3-trifluoromethylbenzyl)hydroxyphosphinyl]ethyl]pentanedioic acid;
- 2-[1-[(2-fluorobenzyl)hydroxyphosphinyl]hexyl]pentanedioic acid;
- 2-[1-[(pentafluorobenzyl)hydroxyphosphinyl]heptyl]pentanedioic acid;
- 10 2-[1-[(2-pyridyl)hydroxyphosphinyl]ethyl]pentanedioic acid;
- 2-[1-[(3-pyridyl)hydroxyphosphinyl]propyl]pentanedioic acid;
- 2-[1-[(4-pyridyl)hydroxyphosphinyl]butyl]pentanedioic acid;
- 2-[1-[(tetrahydrofuranyl)hydroxyphosphinyl]but-3-enyl]pentanedioic acid;
- 15 2-[1-[(2-indolyl)hydroxyphosphinyl]pentyl]pentanedioic acid;
- 2-[1-[(3-indolyl)hydroxyphosphinyl]hexyl]pentanedioic acid;
- 2-[1-[(4-indolyl)hydroxyphosphinyl]heptyl]pentanedioic acid;
- 2-[1-[(4-indolyl)hydroxyphosphinyl]-1-fluoromethyl]pentanedioic acid;
- 20 2-[2-[(2-thienyl)hydroxyphosphinyl]propyl]pentanedioic acid;
- 2-[1-[(2-thienyl)hydroxyphosphinyl]-1-phenylmethyl]pentanedioic acid;
- 2-[1-[(3-thienyl)hydroxyphosphinyl]ethyl]pentanedioic acid;
- 25 2-[1-[(4-thienyl)hydroxyphosphinyl]propyl]pentanedioic acid;
- 3-[(2-pyridyl)hydroxyphosphinyl]-3-prop-1-enyl-2-phenylpropanoic acid;
- 3-[(3-pyridyl)hydroxyphosphinyl]-3-*t*-butyl-2-phenylpropanoic

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acid;

3-[(4-pyridyl)hydroxyphosphinyl]-3-pentyl-2-phenylpropanoic
acid;

5 3-[(tetrahydrofuranyl)hydroxyphosphinyl]-3-hexyl-2-phenyl
propanoic acid;

3-[(tetrahydrofuranyl)hydroxyphosphinyl]-3-fluoro-2-phenyl
propanoic acid;

3-[(2-indolyl)hydroxyphosphinyl]-3-t-butyl-3-hexyl-2-phenyl
propanoic acid;

10 3-[(2-indolyl)hydroxyphosphinyl]-2,3-diphenylpropanoic acid;

3-[(3-indolyl)hydroxyphosphinyl]-3-methyl-2-phenylpropanoic
acid;

3-[(4-indolyl)hydroxyphosphinyl]-3-ethyl-2-phenylpropanoic
acid;

15 3-[(2-thienyl)hydroxyphosphinyl]-3-propyl-2-phenylpropanoic
acid;

3-[(3-thienyl)hydroxyphosphinyl]-3-prop-2-enyl-2-phenyl
propanoic acid;

20 3-[(4-thienyl)hydroxyphosphinyl]-3-t-butyl-2-phenylpropanoic
acid;

3-(benzylhydroxyphosphinyl)-3-pentyl-2-(2-pyridyl)propanoic
acid;

3-(benzylhydroxyphosphinyl)-3-hexyl-2-(3-pyridyl)propanoic
acid;

25 3-(benzylhydroxyphosphinyl)-3-fluoro-2-(3-pyridyl)propanoic
acid;

3-(benzylhydroxyphosphinyl)-3-pentyl-3-hexyl-2-(4-pyridyl)
propanoic acid;

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3-(benzylhydroxyphosphinyl)-3-phenyl-2-(4-pyridyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-methyl-2-(tetrahydrofuranyl)propanoic acid;

5 3-(benzylhydroxyphosphinyl)-3-ethyl-2-(2-indolyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-propyl-2-(3-indolyl)propanoic acid;

10 3-(benzylhydroxyphosphinyl)-3-prop-1-enyl-2-(4-indolyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-t-butyl-2-(2-thienyl)propanoic acid;

3-(benzylhydroxyphosphinyl)-3-pentyl-2-(3-thienyl)propanoic acid;

15 3-(benzylhydroxyphosphinyl)-3-hexyl-2-(4-thienyl)propanoic acid; and

a pharmaceutically acceptable salt, hydrate, or a mixture thereof.

20 7. A pharmaceutical composition comprising an effective amount of a compound of claim 6 and a pharmaceutically acceptable carrier.

8. A method of treating cancer in an animal comprising administering to said animal an effective amount of a compound of claim 6.

25 9. The method of claim 8 wherein the compound is administered in combination with an additional therapeutic agent selected from the group consisting of: therapeutic hormones, chemotherapeutic hormones, anti-angiogenesis agents,

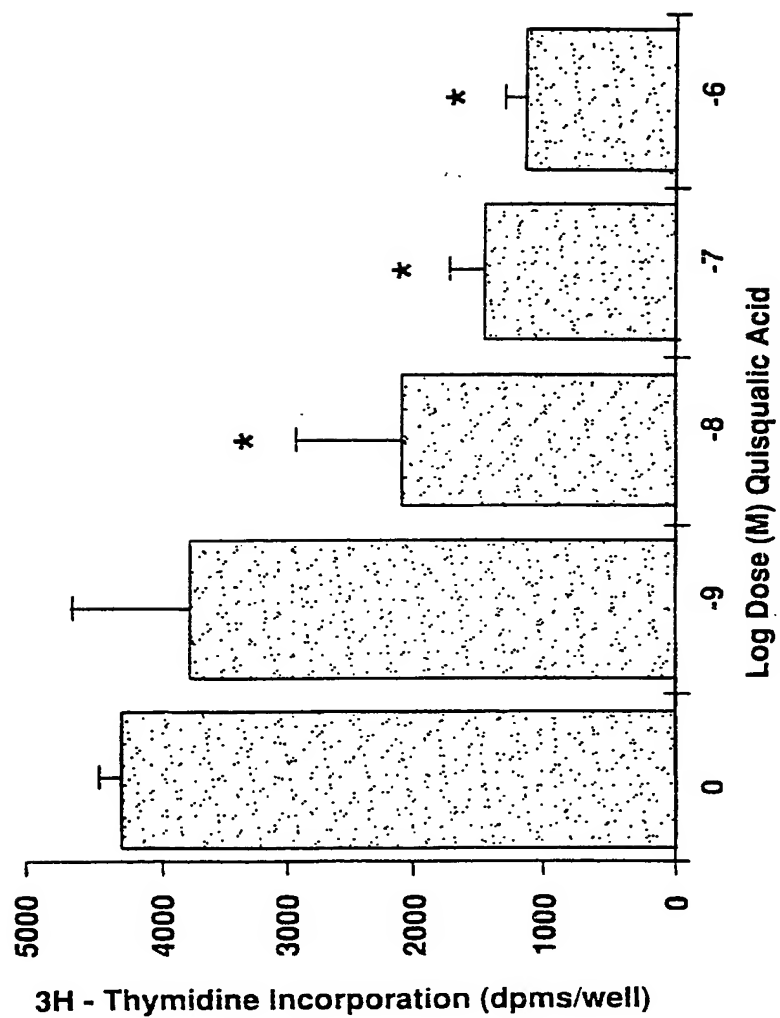
-118-

radiolabelled compounds, and mixtures thereof.

10. The method of claim 8 wherein the cancer is selected from the group consisting of: brain cancer, cancer of the adrenal cortex, kidney cancer, testicular cancer, benign
5 prostatic hyperplasia, and prostatic adenocarcinoma.

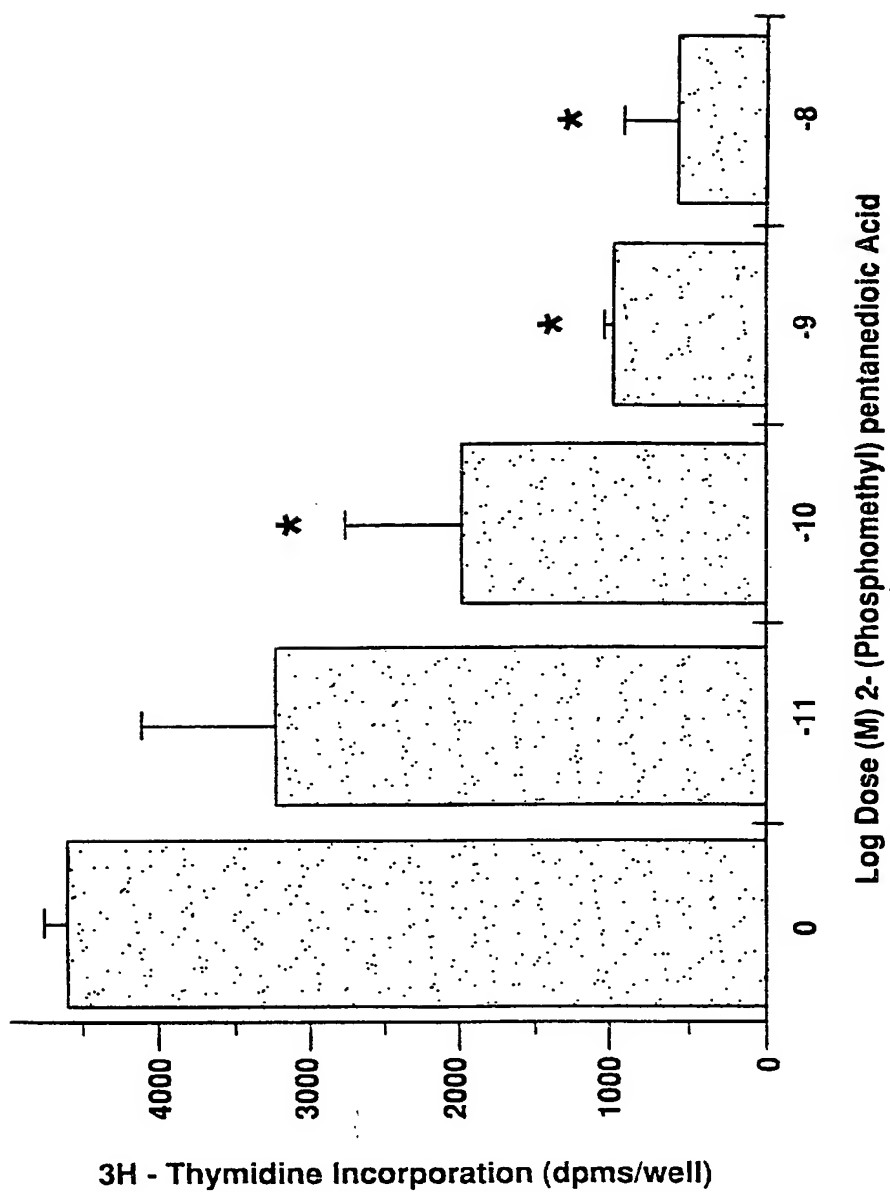
1 / 6

FIG.1



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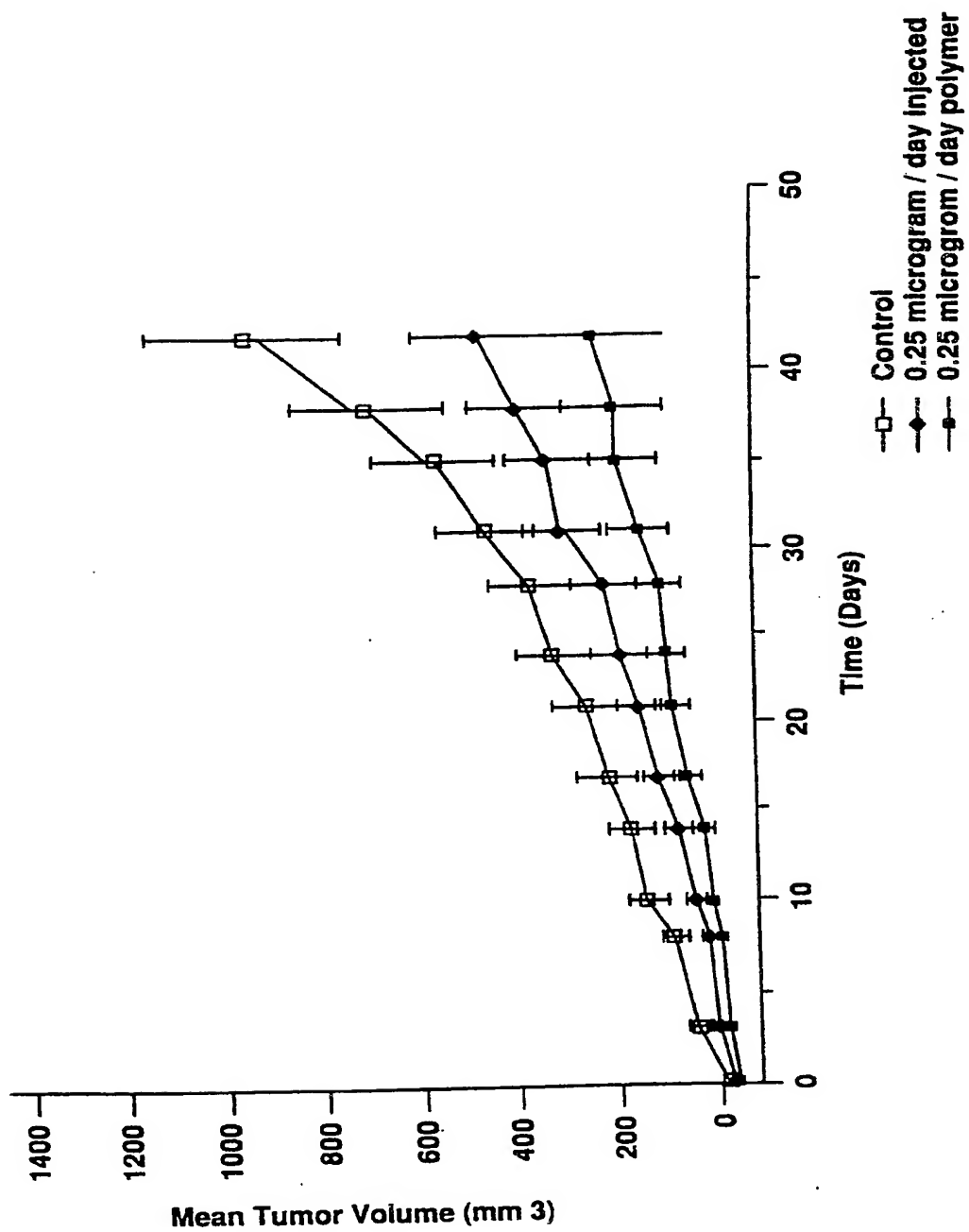
FIG.2



SUBSTITUTE SHEET (RULE 26)

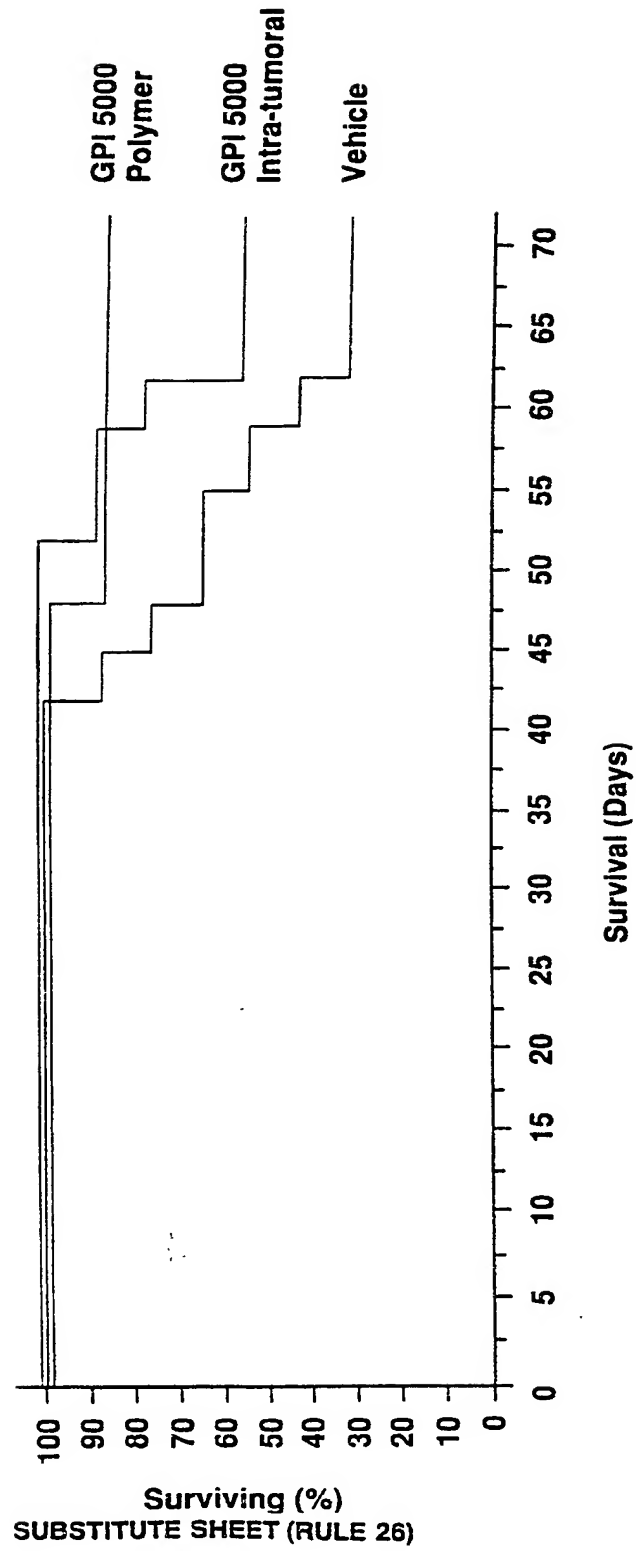
3 / 6

FIG.3

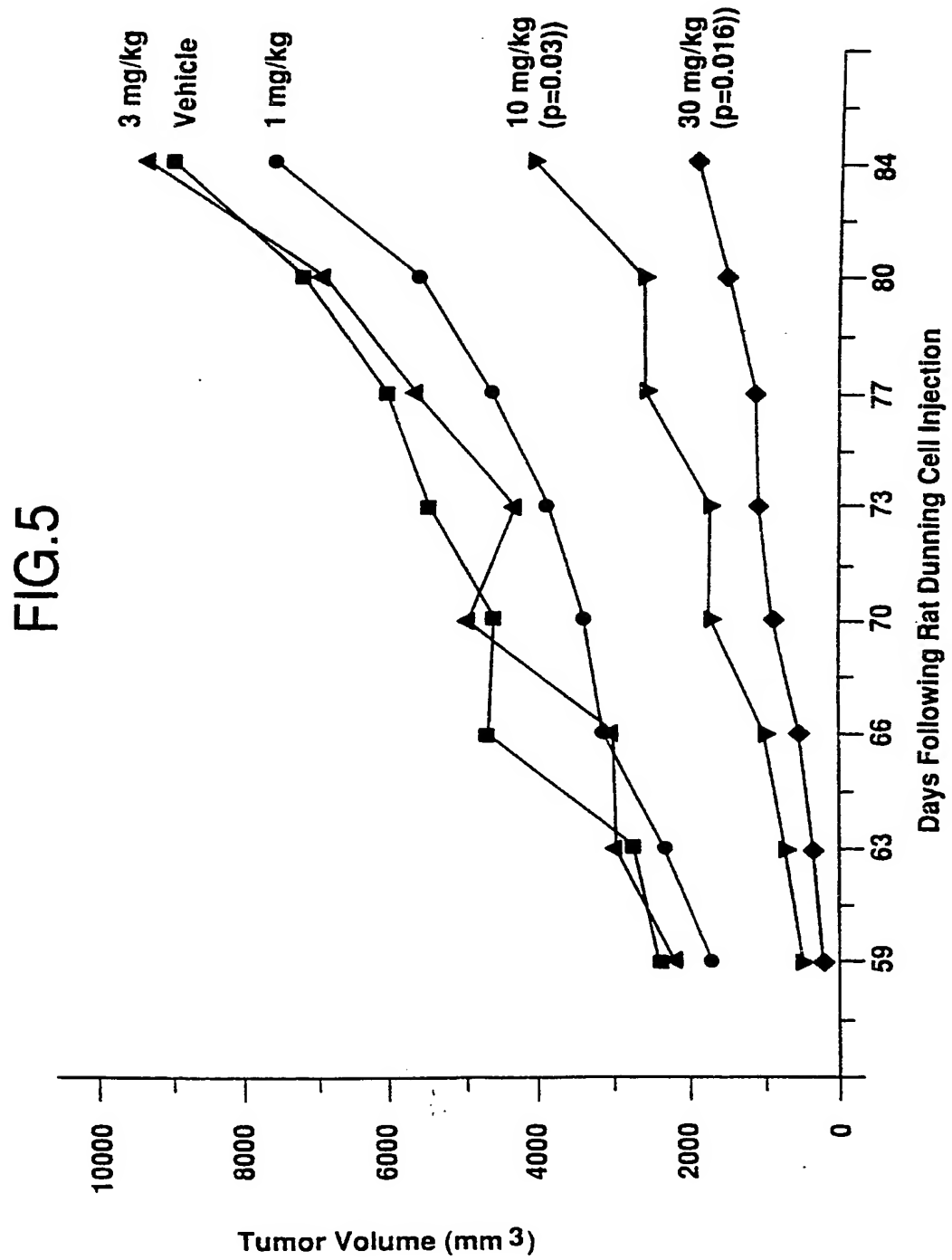


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FIG.4



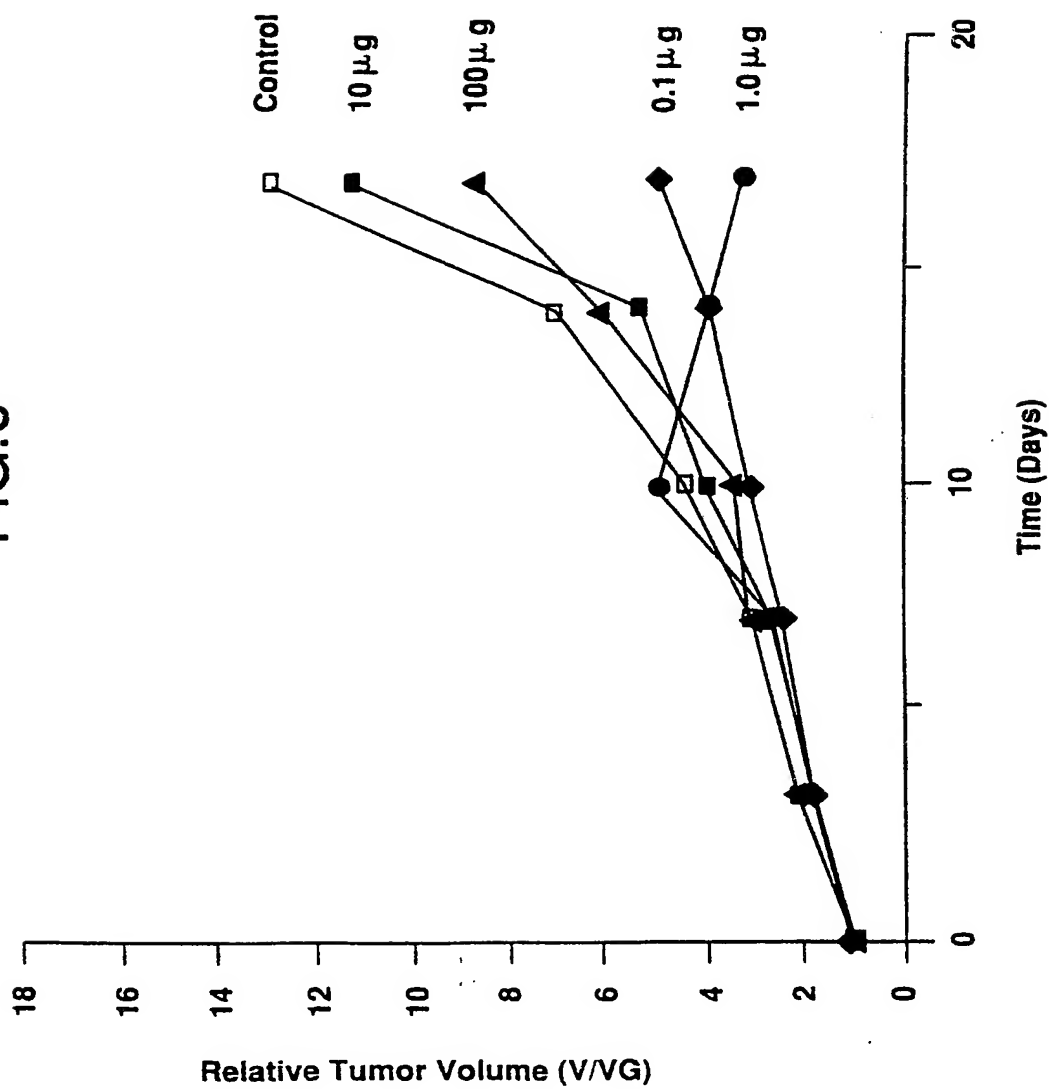
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SUBSTITUTE SHEET (RULE 25)

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FIG.6



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14347

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) :Please See Extra Sheet. US CL :Please See Extra Sheet. According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/109, 336, 415, 438, 461; 548/469; 549/79, 501; 546/341; 562/24 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, CAS ONLINE, MEDLINE search terms: Naaladase inhibitors, cancer, prostate cancer		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,602,092 A (THOTTATHIL et al) 22 July 1986, claim 1.	1,2,6,7
A	US 4,988,681 A (ISHIKAWA et al) 29 January 1991, claim 1.	1,2,6,7
A	JACKSON, P.F. Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase N-acetylated alpha-linked acidic dipeptidase. J. Med. Chem. 1996, volume 39, pages 619-622, especially compound 7 on page 620.	1,2,6,7
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *&* document member of the same patent family		
Date of the actual completion of the international search 16 SEPTEMBER 1997		Date of mailing of the international search report 13 NOV 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer CHANA AULAKH Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14347**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14347

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/34, 31/38, 31/44, 31/66, 31/405; C07F 9/22; C07D 209/04, 213/16, 307/34, 333/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/109, 336, 415, 438, 461; 548/469; 549/79, 501; 546/341; 562/24

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

I. Compounds of formula I where R1, R2, R3 or R4 is not a furyl, thienyl, indolyl or pyridyl, classifiable in class 562, subclass 24.

II. Compounds of formula I where R1, R2, R3 or R4 is a furyl, classifiable in class 549, subclass 501.

III. Compounds of formula I where R1, R2, R3 or R4 is a thienyl, classifiable in class 549, subclass 79.

IV. Compounds of formula I where R1, R2, R3 or R4 is an indolyl, classifiable in class 548, subclass 469.

V. Compounds of formula I where R1, R2, R3 or R4 is a pyridyl, classifiable in class 546, subclass 341.

The claims are deemed to correspond to the species listed above in the following manner:

Species I : claims 1-10.

Species II : claims 1-10.

Species III : claims 1-10.

Species IV. : claims 1-10.

Species V. : claims 1-10.

The following claims are generic: claims 1-10.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: There is no common core Which in the Markush Practice, is a significant structural element shared by all the alternatives; see PCT Administrative Instructions Annex B Part I (f) (i) (B) (1) and further, all alternatives do not belong to a recognized class of chemical compounds in the art to which the invention pertains; see supra (B) (2).

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